The Utility of Caffeine as an Attentional Enhancer

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Abstract

Caffeine is the most frequently consumed stimulant worldwide and has been championed as an attentional enhancer in clinical trials for over a hundred years. There is broad agreement that caffeine induces an attention enhancing effect. However, a minority disagree and propose the caffeine withdrawal reverse hypothesis. This posits that, due to inadequate caffeine withdrawal procedures in study design, the beneficial properties displayed by caffeine on attention result from reversal of caffeine withdrawal. In caffeine studies with an appropriate withdrawal period prior to intervention, no clear beneficial effect on attention has been demonstrated. This thesis critically appraised the literature and using a novel experimental paradigm, explored the utility of caffeine as an attentional enhancer in participant groups consisting of healthy elderly, dementia with Lewy bodies (DLB), Parkinson's disease (PD) and multiple sclerosis (MS) sufferers.

I conducted a blinded, randomised controlled, cross over design trial to explore whether caffeinate improved performance on experimental and real-world tasks of attention. I systematically assessed three broad areas of attention: alerting, orienting and executive networks with neuropsychometry tasks aligned to each domain. These experiments are unique within the literature as they combine a complete caffeine withdrawal period prior to intervention, a systematic approach to assessing for attentional enhancement and patient cohorts not previously investigated in relation to the attentional effect of acute caffeine ingestion.

I conclude caffeine is not an effective attention enhancer, at least not in populations of healthy older people or people with PD or DLB. The possibility remains that caffeine may enhance attention in people with MS and perhaps in other situations such as sleep deprivation.

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I would like to thank my two supervisors Liz Coulthard and Pat Kehoe for their supervision during my PhD. Liz in particular has provided patient support and invaluable advice throughout every step of my PhD journey and without her I would not have made it this far.

Author’s Declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

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Chapter 1

Introduction

This chapter is based on published work:
Sharma, K., Davis, T., & Coulthard, E. (2016). Enhancing attention in neurodegenerative diseases: current therapies and future directions. Translational Neuroscience, 7(1), 98-109.
I have only included parts of the manuscript I personally wrote, I have not included text written by T Davis. E Coulthard contributed in a supervisory role only.

Cognitive impairment, especially dementia is a health predicament growing exponentially in response to an ageing population. Research in this area has historically been poor compared to other diseases such as stroke, diabetes and cancer. However, in 2015, the then Prime Minister David Cameron, set out the "Challenge on Dementia 2020' to redress this issue (Health, 2015). The financial burden associated with dementia such as increased home care packages or nursing home placements, are estimated to outweigh the costs of stroke, heart disease or cancer, the three most common causes of premature death in the UK (Dowrick and Southern, 2014). This is a problem not just limited to Britain but a global health issue, as the number of people living with dementia worldwide is projected to double over the next 15 years (Prince et al., 2015).

A key objective of the 2020 directive was to increase research into dementia treatments. At present there are no disease modifying medications but there are two classes of drugs licensed as symptomatic treatments; cholinesterase inhibitors (donepezil, rivastigmine, galantamine) and glutamate NMDA receptor antagonists (memantine) (National Institute for Clinical Excellence, 2011). Cholinesterase inhibitors increase the circulating levels and

hence action of the neurotransmitter acetylcholine, which mediates cognitive enhancement through improved attention (discussed below). The success of this treatment grants a unique opportunity to repurpose established stimulants, such as caffeine, as attentional enhancers, with the potential to improve the health of cognitively impaired people and reduce the burden of care costs.

This thesis will focus on the influence of caffeine on attention in health and cognitively impaired populations. It will start with a review of caffeine pharmacology and frame it within a neurobiological model of attention.

1.1 Caffeine

Caffeine is the most commonly ingested psychostimulant with over 80% of the world's population estimated to consume caffeine daily (Barone and Roberts, 1996). The origins of therapeutic caffeine use can be traced back to 11th century Ethiopia. According to myth, Kaldi, a goat herder noticed that after eating berries from a certain tree, his goats became restless and energetic. He made a drink from the beans, which he found kept him alert and shared this with his local community. As news of the energising drink spread, in order to protect trade in the berries, they were roasted to prevent identification, and to this day coffee beans are still roasted (Pendergrast, 2010). It was not until 1819 when the active ingredient was first chemically isolated and subsequently synthetically produced in 1895 following which it has been used to fortify beverages and over the counter medication (Waldvogel, 2003).

100 years ago the psychologist Hollingworth undertook the first human psychopharmacological study of caffeine (Hollingworth, 1912), commissioned by the Coca-Cola Company in response to a lawsuit accusing it of intentionally adding caffeine, perceived by some as harmful, to its products (Benjamin et al., 1991). At that time Coca-Cola marketed their drink as "invigorated the fatigued body and quickened the tired brain." However, the chemist in the U.S. Department of Agriculture who instigated the trial, viewed caffeine as a poison

and habit forming drug, and was unhappy at its sale to children. Hollingworth's work demonstrated increased psychomotor speed following caffeine ingestion with possible enhancement of cognitive performance. In the intervening century the understanding of cognitive function especially attention has grown exponentially in response to improved imaging techniques, however, despite its ubiquity the understanding of how caffeine affects cognition is relatively incomplete (Nall et al., 2016).

1.2 Attention

Attention facilitates cognitive functions such as memory, language, problem solving, perception optimal for goal orientated behaviour. The ambient environment is a constant source of sensory stimulation in the form of sights, sounds, taste, temperature and touch. To actively process all these stimuli continuously would be unnecessarily demanding upon a finite cognitive resource, as much of the information would be irrelevant to the task at hand. A crucial cognitive skill for survival is the ability to selectively process or disregard information from the abundance of sensory input enabling goal directed behaviour to be achieved. The importance of attention is often overlooked as it does not localise anatomically and is therefore difficult to study, however, when impaired the consequences can be devastating. This is evident in many neurological diseases such as dementia with Lewy bodies, where people can suffer with fluctuations in attention lasting minutes to days rendering them confused and unable to interact effectively with the word around them (Boot, 2015).

Attention describes a complex interaction of multiple independent systems distributed within the brain (Fan et al., 2005a, Pessoa et al., 2003). Voluntary "top-down" shifts of attention are goal directed, driven by information regarding the current task whilst automatic "bottom-up" exogenous influences of attention are stimulus driven (Buschman and Miller, 2007). Through both top-down and bottom up influences, attention allows us to selectively process or

inhibit information from the abundance of sensory input over multiple domains (Treisman and Gelade, 1980, Treisman, 1998). Breakdown of specific brain areas or neurotransmitter systems causes selective disruptions of attentional networks in both healthy aging and disease processes (Coulthard et al., 2006). Thus attention can be considered a bottle neck for cognitive processing (Kahneman, 1973) – enhance attention and overall brain function can be improved.

Whilst there is clearly an overlap, reduced sleepiness should not be interpreted as increased alertness, the former is measured by sleep pattern or assessing daytime fatigue whilst the latter is measured by tests of attention. In fact during sleep one is still attending to the environment, which is why you would wake up if threatening stimuli were sensed such as a change in temperature or a startle reflex to loud sound. Therefore attention should be considered a constant cognitive process, which shifts from being goal directed during wakefulness, to bottom up influenced during sleep.

1.2.1 A brief history of attention

At the turn of the 20th century the domain of attention was described as the centrepiece of the psychological enterprise (Posner and Rothbart, 2007), however, research in the field did not blossom until after World War Two when it was recognised that information processing in military tasks were underpinned by attention, such as air traffic controllers looking at radar for prolonged periods of time or being able to multitask when receiving competing messages from incoming and departing aircraft. The UK psychologist Broadbent was the first to propose a filter model also termed *selective attention,* with a cognitive bottleneck, based in his work from the "dichotic listening task" (Broadbent, 1958). In this task participants were simultaneously given different numbers to each ear and then asked to recall what they heard. Participants typically recalled information most accurately from only one ear and ignored or were less able to identify information from the other ear, when presented at the same time. Broadbent suggested a sensory filter diverted attention to a singular

sensory stimulus e.g. to the left ear, and blocked out other sensory stimuli e.g. the right ear. This was a necessary process to prevent our limited capacity to process information from becoming overloaded – the so called cognitive bottle neck. This model has been revised over the years, notably by Treisman who proposed, in contrast to Broadbent's all or nothing filter, attention as an attenuator which can prioritise and switch between stimuli (rather than simply ignore them) depending on the context/importance (Treisman, 1964). This model was further supported by MacKay who demonstrated that unattended stimuli were still cognitively processed and could influence the perception of attended stimuli (Mackay, 1973).

Once it became established that one could attend to more than one stimulus at a time, Kahneman developed the notion of *divided attention*, proposing a central processor that would evaluate and actively allocate attention according to task demands (Kahneman, 1973). This theory was further developed by Schneider & Shiffrin to allow a distinction between controlled and automatic processing (Shiffrin and Schneider, 1977). Automatic processing required no *active* allocation of attention whereas controlled processing requires deliberate allocation of attention to a task. A limitation of this theory was the lack of mechanism for controlled processing to become automatic. Interestingly automatic processing is associated with greater errors of task completion as the fast processing reduces the ability for error monitoring (Reason, 1992).

Sustained attention is the ability to maintain focus on specific stimuli for a prolonged period of time to enable task completion, often used interchangeably with vigilance (Sarter et al., 2001). Along with selective and divided attention, sustained attention formed the basis of a functional concept of attention, which was prevalent until the 1990s. The advent of structural and subsequently functional imaging allowed a physiological classification to emerge based on neurotransmitter networks and anatomical regions of interest (Petersen and Posner, 2012).

Corbetta and Shulman proposed a dual attentional network consisting of two distinct functional and anatomical systems (Corbetta and Shulman, 2002). The ventral attention network is predominantly dominant to the right hemisphere, inferior frontal cortex and temporoparietal cortex. It is associated with shifts of attention towards behaviourally relevant stimuli especially when unexpected or unattended. The complimentary dorsal attention network is bilateral and comprises the intraparietal cortex and superior frontal cortex. It is associated with voluntary orienting of attention, which is enhanced by the presentation of cues. The two systems are proposed to work in union with the ventral attention network functioning as a circuit breaker to the dorsal attention network (Corbetta et al., 2008, Vossel et al., 2014).

Yu and Dayan developed an exploitation versus exploration model of attention based on acetylcholine and norepinephrine respectively. Exploitation being a state of expected uncertainty and exploration being related to unexpected uncertainty. Cholinergic neurons in the nucleus basalis, have heterogeneous behaviours (Gu, 2002) in contrast to the more homogeneous activity of norepinephrine neurons in the locus coeruleus (Aston-Jones and Bloom, 1981). Yu and Dayan describe a complex interaction whereby acetylcholine can antagonise or synergise norepinephrine depending on the degree to exploitation required (Yu and Dayan, 2005).

A "multiple demand system" has been proposed to allow dynamic control of complex cognitive processing to be organised into smaller, less demanding series of attentional events. Anatomically this localises to multiple region within the frontal and parietal lobe with accompanying activity in the basal ganglia, thalamus and cerebellum. There is consistency of topographical areas of cortical activation within the multiple demand systems but the nature of the cognitive task alters the degree of activation within a particular area (Duncan, 2013). This model offers a fluid mechanism for attention within executive processing.

After careful consideration this PhD thesis has selected the Posner-Petersen model of attention as the foundation on which to ascribe the attentional

effects of caffeine. Whilst contemporary theories all demonstrate merit, and no singular model is without shortcoming, Posner's model offers a clear framework combining neuropsychological function with neuroanatomical structure which allows specific facets of attention to be selectively probed. This model is distinguished by its comprehensive approach which I feel is unmatched by the contemporary models described above. It is explored in more detail below.

1.2.2 Neurobiology of attentional networks: the Posner-Petersen model

Anatomical explanations of attention involve a trinity of independent but interacting core networks, each with its own characteristic psychological and neuroanatomical properties; the *alerting*, *orienting*, and *executive* networks of attention (Petersen and Posner, 2012). Their nuclei emanate from the brainstem, as part of the ascending reticular activating system and pathways diffusely disseminate within the cerebrum to synchronise large areas of cortical activity (Robbins, 1997).

The alerting network characterises the ability to maintain optimal vigilance and performance during a task, it is not just the readiness to receive information (exploration) that is being enhanced but also the readiness to respond to stimuli (exploitation). This relies on a right hemisphere cortical and subcortical network involving the anterior cingulate cortex as a synchronizing structure (Mottaghy et al., 2006). Frontal, thalamus, amygdala and parietal regions are particularly active during tasks of alerting attention (Fan et al., 2005a). The neurotransmitter norepinephrine (NE) arising in the locus coeruleus in the midbrain, is the sole source of NE neurons in the brain (Sara, 2009) and has been implicated in the alerting network, notably in its ability to elevate readiness to respond as a result of an external cue (Aston-Jones and Cohen, 2005, Witte and Marrocco, 1997, Witte et al., 1997, Coull et al., 2001). Two different modes of NE activity have been proposed, tonic and phasic. Tonic activity occurs when exploration is the dominant requirement and is associated with disengaging from the current cue, searching for new behaviours and transitioning to a new cue. Phasic activity occurs when exploitation is required

and is associated with behaviours that optimise task performance (Aston-Jones and Cohen, 2005). Alerting should be considered a prerequisite for other attention networks to function, one can expect tonic NE activity during orienting attention network tasks and phasic NE activity during executive attention network tasks (discussed below).

The orienting network is concerned with the ability to align attention to a source of sensory input both overtly, in conjunction with eye movements, or covertly, in the absence of eye movements. Cholinergic cells project from their origin in the laterodorsal tegmentum and pedunculopontine nuclei to either (i) the thalamus through the dorsal tegmental pathway or (ii) the corticopetal basal forebrain system and cortex via the ventral tegmental pathway (Inglis and Winn, 1995). Unlike brainstem NE cells, cholinergic neuron activity is not stimulus specific and does not alter with novel stimuli. The orienting of attention uses a network including the superioparietal cortex, temporoparietal cortex, frontal eye fields, pulvinar, and superior colliculus (Corbetta and Shulman, 2002, Petersen and Posner, 2012). Furthermore, impairments to orienting tasks were found following lesions to the basal forebrain systems of macaque monkeys (Voytko et al., 1994), implicating these areas in the orienting network. Orienting has been linked to activation of cholinergic pathways (Beane and Marrocco, 2004), supported by research in rat brains that suggest acetylcholine, but not dopamine, is important for orienting tasks (Everitt and Robbins, 1997).

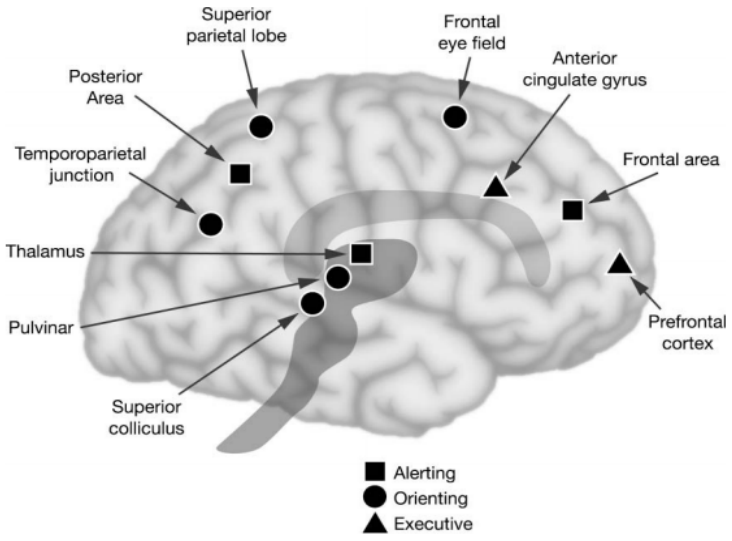

Figure 1.1 Anatomy of the trinity of attention networks: alerting, orienting, and executive (Posner and Rothbart, 2007)

Executive networks are called upon during tasks that require top-down attentional control and the ability to focus attention selectively according to task demands. Tasks involving selective planning, monitoring or inhibition of automatic responses produce subjective reports of mental exertion. During attention that is mentally exerting and conflict monitoring, the anterior cingulate cortex is consistently activated (Botvinick et al., 2004). Interestingly, this network may possess higher-level metacognitive properties, in other words, the network might be involved in generating the subjective impression of cognitive effort (Fernandez-Duque et al., 2000, Fernandez-Duque and Thornton, 2000). It dynamically interacts with primary sensory regions via bottom-up signals, which subsequently enhance top-down modulation of sensory processing via a feedback mechanism (Crottaz-Herbette and Menon, 2006). Anatomically the network of structures involved in executive attentional tasks includes the anterior cingulate cortex (Botvinick et al., 2001), the medial frontal cortex (Petersen and Posner, 2012), lateral ventral prefrontal cortex, and basal ganglia. The influence of the mesocortical dopamine system on these areas implicates the neurotransmitter dopamine in executive attention.

Terminology		Neurotransmitter systems predominantly implicated	Common cognitive tests
New	**Old**		
Alerting network	Sustained attention	Norepinephrine	Cognitive reaction time
Orienting network	Selective attention	Cholinergic	Rapid Serial Visual Presentation paradigm
Executive network	Divided attention	Dopamine	Stroop task, Wisconsin Card Sorting Test

Table 1.1 Attentional domain classification

The Posner-Petersen model of attention assigns individual attentional networks to singular neurotransmitter systems however the reality is more complex. Both the Corbetta and Shulman, and Yu and Dayan models of attention describe interacting networks, which enhance or abate the other, working symbiotically to optimise goal directed attention. Whilst the Posner-Petersen model is not explicit in describing inter-network interactions, intact alerting attention could be considered a prerequisite for other attentional networks to effectively function. The Attention Network Test was originally developed by Posner's group to assess the three attentional networks independently (Fan et al., 2002). However, subsequent analyses have identified multiple network interactions, which allude to a complex interplay of neurotransmitter pathways even when performing seemingly network specific tasks (Callejas et al., 2004, MacLeod et al., 2010).

1.2.3 Optimising attention

As far back as 1908 Yerkes and Dodson recognised performance ability conforming to an inverted U shape (or bell shaped) in the context of arousal (Yerkes and Dodson, 1908). Increases in arousal are associated with an increase in performance up to an optimum point, following which higher levels of arousal only impair performance. When applied to participants consuming a variable range of caffeine doses, their performance on easier cognitive tasks improved as caffeine dosage increased, since easier tasks require greater arousal but on more difficult tasks performance initially improved with dose increase before deteriorating at the highest doses, in keeping with the Yerkes-Dodson law (Anderson, 1994).

When considering caffeine as an attentional enhancer, choice of optimal dose is not straightforward and dependent on task complexity. It is possible that caffeine could improve performance on alerting, orienting and executive attention but optimal performance for each domain would likely require different doses, as exemplified by the psychostimulant modafinil (Wesensten et al., 2002). Overall moderate doses of caffeine are reported to improve attention and processing speed performance whilst high doses (>500mg) typically cause impairments (Brunye et al., 2010, van der STELT and Snel, 1998). Some authors attribute this to caffeine induced increases in anxiety (Lorist and Tops, 2003, Smith, 2002).

A high proportion of studies have investigated caffeine in doses that far exceed levels habitually consumed in foodstuffs, given an instant coffee contains approximately 60mg caffeine and most trial data compares ≥150mg caffeine. Individuals tend to self titrate their caffeine consumption by varying the cups of tea or coffee they consume in a day. Interestingly it appears this self titration averts toxic levels of caffeine ingestion as suggested by experimental data where participants who unknowingly consumed high doses of caffeine added to their coffee, consequentially reduced their daily coffee intake (Griffiths et al., 1986).

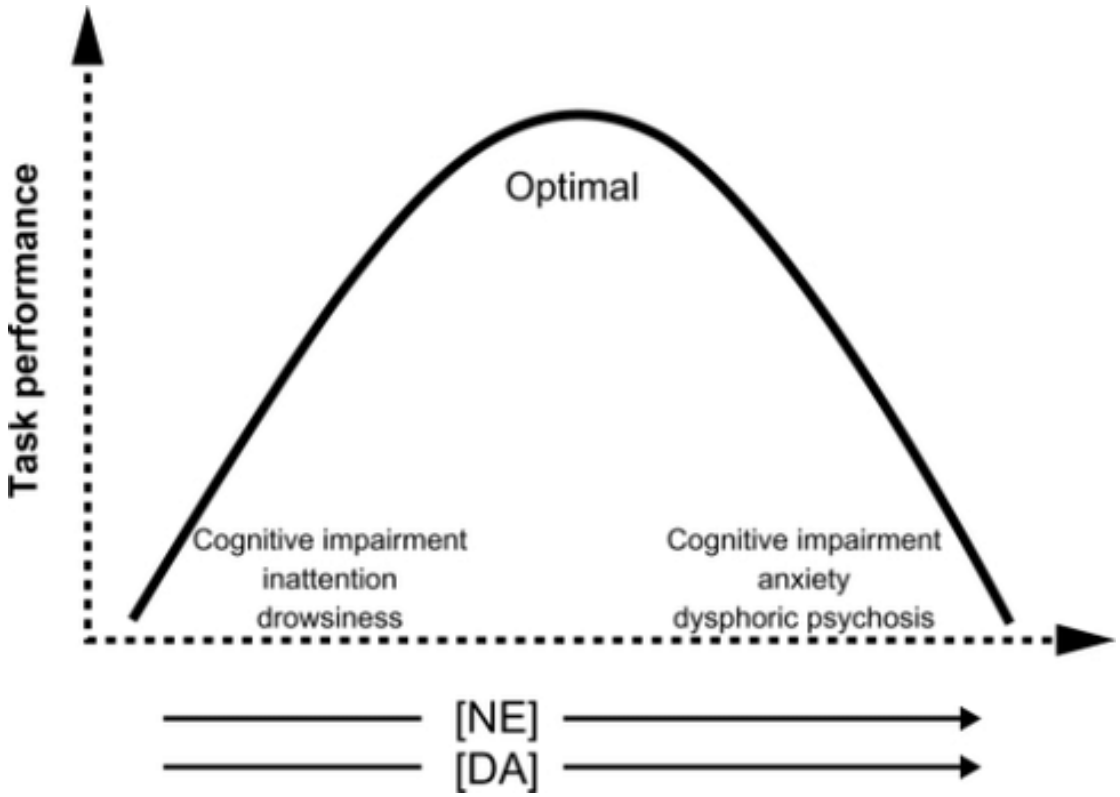

Figure 1.2 The Yerkes-Dodson Law applied to (NE) and dopamine (DA) neurotransmission (Blier and Briley, 2011)

1.3 Caffeine pharmacology

Caffeine (1,3,7-trimethylxanthine) is a plant alkaloid naturally found in coffee, tea, chocolate, guarana, and plants such as the kola nut, and frequently added in its synthetic form to carbonated drinks (Baker et al., 2014). Chemically it is a xanthine derivative, consisting of a purine base, and is related to other xanthines, including aminophylline, pentoxifylline, theobromine and theophylline which all contain similar properties.

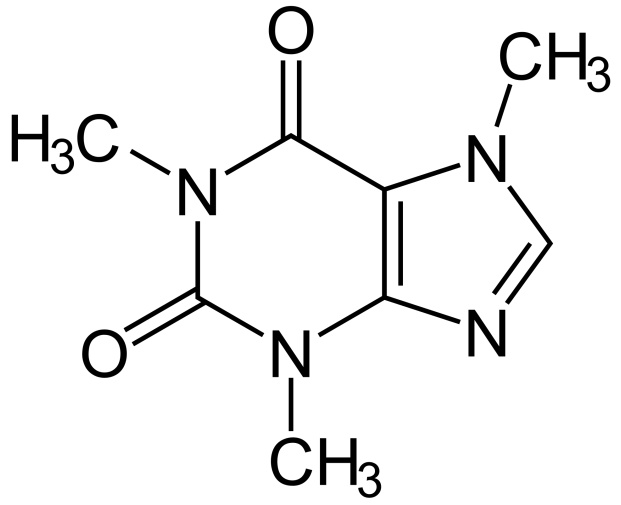

Figure 1.3 Chemical structure of caffeine (adapted from (Mohamed et al., 2013)) Caffeine differs from other xanthine derivatives by the number of methylations (CH_3 groups).

Caffeine is a widely used stimulant that has multiple behavioural and physiological effects (Fredholm et al., 1999) (Glade, 2010), with consumers often citing psychostimulant benefits after use. It easily traverses the blood brain barrier to exhibit its main neurochemical effect on the endogenous neuromodulator adenosine. As a by product of ATP metabolism, adenosine accumulates in the extracellular space around any somatic cell but specifically cerebral glial cell ATP metabolism is hypothesised to function as a homeostatic regulator of cerebral energy expenditure (Halassa et al., 2009).

Of the four types of adenosine receptors, it competes non selectively and antagonistically with high affinity at A1 and A2a (Ferre, 2010), resulting in attenuation of neural activity, inhibiting the function of γ-aminobutyric acid (GABA) neurons and promoting the release of excitatory neurotransmitters such as glutamate, norepinephrine, acetylcholine and dopamine (Koppelstaetter et al., 2008b, Ribeiro and Sebastiao, 2010). This is proposed to elicit a moderate stimulant and mood enhancing effect. In its natural form adenosine accumulates over the course of the day with the effect of slowing down neural activity and increasing sleepiness following prolonged wakefulness (Porkka-Heiskanen et al., 2002). A1 receptors are found in the hypothalamic nuclei, cerebellum, and hippocampus, but are also widely distributed throughout the cerebral cortex (Smith et al., 2005). A2a receptors are concentrated in the striatum and regulate

perfusion by vasodilation, thus inhibiting psychomotor function. The competitive occupation of these receptors by caffeine decreases cerebral perfusion (Addicott et al., 2009, Haase et al., 2005, Chen and Parrish, 2009, Kennedy and Haskell, 2011), increases vasoconstriction, enhances psychomotor function (Ferre, 2010) and facilitates dopamine release at the pre-synaptic membrane, although the exact molecular mechanism is unknown (Ferre et al., 1997).

A1 receptors are considered neuroprotective as they induce inhibitory effects on the nervous system whereas A2a receptors are associated with neurodegeneration as they possess excitatory effects (Cunha, 2005). Caffeine has therefore been proposed to have a neuroprotective mechanism via A2a receptor antagonism (Chen et al., 2001, Stockwell et al., 2017). Interestingly there is a selective loss of A1 but not A2a receptor density throughout the cerebrum with increasing age in healthy individuals (Mishina et al., 2017). I would hypothesise the imbalance A1 to A2a receptors with in healthy aging may predispose to neurodegeneration.

It is worth mentioning the effect of neurological disease on adenosine receptors, specifically diseases that will be used to assess caffeine as an attentional enhancer in the data chapters contained in this thesis. In Parkinson's disease compared to aged matched health controls, there is a decrease in A2A receptors in the caudate nucleus and putamen but an increase in the substantia nigra pars reticulata (Hurley et al., 2000). Adenosine receptor density has not been specifically studied in dementia with Lewy bodies however one could extrapolate from the Parkinson's disease literature as these two conditions are on a spectrum. In secondary progressive multiple sclerosis A2a receptors are increased in normal appearing grey and white matter compared to aged matched healthy controls (Rissanen et al., 2013). Regional or selective adenosine receptor density has not been studied in multiple sclerosis.

Adenosine accumulation is a by-product of neuronal cellular metabolism and has been proposed to function as a homeostatic regulator of energy by promoting sleep (Benington and Heller, 1995). It is present both extra and

intracellularly with extracellular accumulation occurring when cellular energy demands exceed energy production. (Dunwiddie and Masino, 2001, Latini and Pedata, 2001). Adenosine has postsynaptic inhibitory effects on basal forebrain cholinergic neurons and laterodorsal tegmental nuclei cholinergic neurons (Arrigoni et al., 2001). It will therefore inhibit the release of wakefulness stimulating neurons, instead promoting sleepiness (Basheer et al., 2004). It is clear then how caffeine, as an adenosine receptor blocker, will induce a wakefulness effect.

Sleep and wakefulness have been proposed to function in a "flip-flop" circuit where each half of the circuit strongly inhibits the other, creating two stable states i.e. awake or asleep, with an avoidance of intermediate states. Wakefulness is promoted by an ascending arousal system with projections from the posterior hypothalamus and brainstem to the forebrain. The laterodorsal tegmental and pedunculopontine tegmental nuclei project cholinergic neurons to the thalamus and basal forebrain, which then project diffusely to the rest of the cortex to regulate activity (Hallanger and Wainer, 1988). This is complemented by projections running a similar path from aminergic nuclei, which consist of noradrenergic neurons from the locus coeruleus, serotoninergic neurons from the raphé nuclei and histaminergic neurons from the tuberomammillary nucleus. This triad of aminergic neurons inhibit REM sleep (Strecker et al., 2000, Saper et al., 2001).

The flip side of the wakefulness circuit is controlled by a descending sleep-promoting system, which project neurons from the ventrolateral preoptic nucleus (VLPO). These neurons contain gamma-aminobutyric acid (GABA) and col-localised galanin, which inhibits the activity of the locus coeruleus, raphé nuclei, tuberomammillary nucleus, laterodorsal tegmental and pedunculopontine tegmental nuclei (Sherin et al., 1998). Animal studies have demonstrated increased extracellular adenosine levels in the basal forebrain associated with decreased wakefulness and slow wave sleep which is most marked after sleep deprivation (Porkka-Heiskanen et al., 1997). Interestingly, following sleep deprivation there is a sustained rise in adenosine in the basal forebrain neurons but not the VPLO. This anatomical selectivity suggests

adenosine induces a sleep promoting effect through inhibition of basal forebrain ascending projections rather than stimulating the descending sleep-promoting VPLO projections (Porkka-Heiskanen and Kalinchuk, 2011).

Acute administration of caffeine results in non selective antagonism of adenosine receptors (Karcz-Kubicha et al., 2003) and critically produces a different psychostimulant profile to chronic ingestion. Chronic antagonism of adenosine receptors leads to up-regulation of A1 but not A2a receptors, which indicates tolerance to selective psychomotor effects with chronic caffeine use (Ferre, 2008). Cerebral blood flow both at baseline and reduction following caffeine consumption is dependent on the level of habitual caffeine consumption. High consumers exhibit a higher resting cerebral blood flow and a greater reduction in blood flow following caffeine ingestion compared to low consumers (Field et al., 2003). The phenomenon of caffeine improving cognitive function but reducing cerebral blood flow runs counter to neurovascular coupling, the principle upon which fMRI interpretation is based. This is the concept of increases in cerebral blood flow running parallel to increases in neuronal activity to ensure the metabolic demands of cerebral activity are met.

Animal studies have shown that chronic caffeine ingestion is not associated with complete tolerance to the sleep reducing effects (Nall et al., 2016). This has been linked to an indirect effect of dopaminergic signalling with animal models demonstrating a rise in cerebral dopamine levels (Solinas et al., 2002). This mechanism is supported by human findings where expressing a lower level of the dopamine transporter is associated with sensitivity to caffeine (Holst et al., 2014). More recently caffeine's antagonism of adenosine receptors have been found via cyclic AMP second messenger systems, to delay the sleep phase of the circadian rhythm by affecting the suprachiasmatic nucleus and melatonin production (Burke et al., 2015). The wakefulness properties are in part dependent on the time of caffeine ingestion with greatest efficacy prior to melatonin secretion i.e. during the day rather than the middle of the night when melatonin levels peak (Wright Jr et al., 1997). This raises the possibility of a dual

mechanism to increase arousal, by increasing synthesis of the wake promoting dopamine and decreasing the biological sedative effects of melatonin.

A large body of work has suggested that even low doses (20 and 30mg) of caffeine improve performance on tests of attention as soon as 20 minutes after consumption (Smit and Rogers, 2000, Lieberman et al., 1987, Hewlett and Smith, 2007). Within 1 hour of oral consumption caffeine is fully absorbed from the gastrointestinal tract (Mumford et al., 1996), reaching peak plasma levels between 30 and 60 minutes (Magkos and Kavouras, 2005, Benowitz, 1990). The half life of caffeine is between 3 and 6 hours with significant intra and inter-individual variability (Balogh et al., 1992). Whilst the recommended maximum intake of caffeine are 100-200 mg, up to four times in a 24 hour period (European Food Safety Authority, 2015), consumption of 150mg can enhance performance for up to 10 hours (Institute of and Committee on Military Nutrition, 2001), potentially making a once daily regime permissible. Advancing age can affect the psychomotor response to drugs as demonstrated with benzodiazepines (Swift et al., 1985a, Castleden et al., 1977), whether this is true with caffeine has not been quantified. Smoking is known to reduce the half life of caffeine (Hart et al., 1976). Caffeine is metabolised in the liver by the cytochrome P450 enzyme CYP1A2, which has a lower activity in women than men (Bebia et al., 2004).

High habitual caffeine consumers may require a greater caffeine dose to experience the same benefits as low habitual caffeine consumers, as animal studies have demonstrated an increased density of adenosine receptors in the brains of habitual caffeine users i.e. incomplete tolerance develops (Fastbom et al., 1990, Varani et al., 1999). In addition to potential cognitive and motor benefits, chronic caffeine ingestion increases plasma adenosine levels which may be neuroprotective (Fredholm et al., 2005), by preventing cellular energy depletion associated with sustained cerebral activity, by promoting sleep and consequentially decreasing the burden of neuronal activity (Porkka-Heiskanen et al., 2002). However, longitudinal data has not demonstrated caffeine to be

protective against later life cognitive decline (Panza et al., 2015, Solfrizzi et al., 2015).

It has been suggested that dependence may occur with just 100mg caffeine per day (James, 1997, Evans and Griffiths, 1999), although caffeine's effect is likely to be dependent on user size as it has a high volume of distribution. Controversy around caffeine's purported stimulant properties has arisen, however, owing to the failure to take account of withdrawal effects. With frequent repeated exposure to caffeine complete tolerance to the alerting effects develop due to changes in adenosine signalling (Zwyghuizen-Doorenbos et al., 1990). With novel high does (1200mg a day) tolerance can develop within one week whilst chronic use with the equivalent of 3 instant coffees a day (100 mg of caffeine) can lead to physical dependence and consequential withdrawal symptoms following abstinence (Evans and Griffiths, 1999).

Potentially debilitating withdrawal symptoms (Juliano and Griffiths, 2004) such as lowered alertness, headache, insomnia, irritability and lethargy begin 12 to 24 hours after abstinence, peak between 20 and 51 hours after abstinence, and vary in severity depending on the regular level of consumption (Rogers et al., 2010). Typically withdrawal symptoms last between 2 and 9 days (Juliano and Griffiths, 2004), this is the time required for cerebral adenosine receptors to restore back to pre-caffeine levels (Ribeiro and Sebastiao, 2010). Interestingly, studies that take withdrawal into account have found caffeine merely restores cognitive performance during withdrawal up to the level of, but not above, baseline (Rogers et al., 2010).

1.4 Physical effects of caffeine

Caffeine benefits the physical performance of regular consumers and naïve consumers alike (Graham, 2001). The Institute of Medicine suggest a caffeine dose of 150mg influences physical performance for up to 10 hours (Institute of Medicine Committee on Military Nutrition, 2001) and the International Olympic Committee prohibit its use above urinary caffeine

concentrations greater than 12 mcg per mL, at which point ingestion is thought to be deliberately for performance enhancement (Jenkinson and Harbert, 2008). However, improved physical performance is not thought to be due to enhanced attention but instead mediated via ergogenic effect on aerobic performance, augmenting lipolysis for muscle metabolism (Magkos and Kavouras, 2004, Davis and Green, 2009).

Caffeine has been consumed for thousands of years and habitual consumption of up to 1000 mg a day poses no risks to human health (Bonita et al., 2007). Acute intake of higher doses may cause tachycardia and has been associated with arrhythmia in individuals with cardiac abnormalities. There are also less serious toxic effects such as restlessness, anxiety, insomnia and nausea. Moderate chronic caffeine consumption may be beneficial with habitual consumption of up to 400 mg a day decreasing the risk of dying from any cause by 10% (Paganini-Hill et al., 2007), although association is not causation.

There are numerous studies in the literature examining adverse cardiovascular effects and there is now consensus that tachyphylaxis develops with habitual caffeine consumption i.e. the recurrent consumption of a pharmacologically active substance results in a diminished effect. The available evidence shows that chronic use of caffeine has no effect on blood pressure that persists beyond 2 weeks (Food and Drug Administration, 2004).

1.5 Attentional network enhancement by caffeine

As discussed above, there are three main neurotransmitters involved with attention: norepinephrine regulates the alerting network, acetylcholine the orienting network and dopamine the executive network.

Caffeine could improve alerting attention due to its noradrenergic effects with doses as little as 2mg/kg able to able to double serum adrenaline concentration compared to placebo 100 minutes following ingestion (Kamimori

et al., 2000). Functional MRI has shown alerting attention to be associated with increased activity in the prefrontal cortex and thalamus, both rich in dopaminergic innervation (Fan et al., 2005b), which has led some researchers to propose dopamine as the primary neurotransmitter responsible for the alerting effects of caffeine(Brunye et al., 2010).

Following caffeine ingestion an increased speed of encoding new information e.g. on testing digit span, is postulated to reflect increased expression of acetylcholine. Working memory has a fixed maximum capacity e.g. 20 seconds, if processing speed is increased then the volume of information that can be processed within that 20 seconds should subsequently increase (Schmiedek et al., 2007). Interestingly episodic memory is not directly enhanced by caffeine (Hewlett and Smith, 2006) and so positive effects of this type are considered to be a result of enhanced orienting attention, improving the ability to concentrate on new or multiple sensory inputs. However, this runs counter to animal studies which demonstrated no increased activity in cholinergic neurons of the basal forebrain and mesopontine tegmentum following caffeine (Deurveilher et al., 2006). Overall there is weak evidence for caffeine enhancement of orienting attention.

The anterior cingulate cortex has been implicated as a vital area for visual executive attention (Bush et al., 2000) and is also strongly innervated by dopaminergic neurons (Lumme et al., 2007). Amplified activity has been demonstrated in these areas on fMRI following caffeine ingestion (Koppelstaetter et al., 2008a). Given caffeine indirectly increases dopamine through adenosine antagonism, caffeine could potentially improve both alerting and executive attention.

For simplicity the psychostimulant effects of caffeine have been explicitly separated into distinct neurotransmitter systems, however, this separation is artificial. To comprehend the complexity of its effect an integrative approach is required, appreciating the interplay between the variable anatomical distribution of cerebral adenosine receptors, the selective A1 receptor up

regulation that occurs with chronic use and the interconnectivity between the various attentional neurotransmitter pathways.

1.6 Caffeine withdrawal controversy

The majority of caffeine studies which demonstrate a beneficial psychostimulant effect of caffeine have crucially not fully withdrawn study participants from caffeine prior to testing (Warburton, 1995). This has led to scepticism of caffeine producing a net benefit to users and the formation of the *caffeine withdrawal reversal hypothesis* (James and Rogers, 2005, Yeomans et al., 2002, Bruce et al., 1991, James, 1998). This asserts caffeine consumed prior to full withdrawal, simply acts to ameliorate the fatiguing effects of withdrawal itself rather than produce an overall, net improvement in cognitive function. It can therefore be deduced that acute caffeine withdrawal (i.e. overnight to 5 days) in habitual consumers will leave them less alert than non-consumers and following ingestion of caffeine, habitual consumers will reinstate to a baseline level of alertness and cognitive function (Heatherley, 2011).

Given few studies use caffeine naive or fully withdrawn participants (despite what they state –with withdrawal too short, typically ranging from overnight to 48 hours), it has not been definitively elucidated whether caffeine has any acute beneficial cognitive effects (Goldstein et al., 1969) other than counteracting sleepiness through adenosine inhibition. Despite these concerns contemporary studies are still being produced without adequately factoring in withdrawal reversal and whilst some show a beneficial effect (Dodd et al., 2015, Bruce et al., 2014, Kamimori et al., 2015) others show a null effect (Ullrich et al., 2015, Rogers et al., 2013).

There are methodological flaws in several studies that report a beneficial effect of caffeine (once the issue of withdrawal reversal has been mitigated) including the potentially incorrect assertion of low consumers as non dependent (Smith et al., 2013) or being classified as non consumers, despite having a

significant salivary caffeine level prior to testing (Haskell et al., 2005). Consuming as little as 100mg caffeine a day for just one week is adequate enough to induce withdrawal symptoms on cessation (Evans and Griffiths, 1999), and this is approximately equivalent to two cups of instant coffee. The lowest dose required to induce dependence and hence withdrawal symptoms on discontinuation, is unknown as research studies have not examined this question using caffeine doses lower than 100mg. However, 25mg of caffeine has been demonstrated to effectively ward off significant withdrawal symptoms in participants were who previously maintained on 300mg per day (Evans and Griffiths, 1999); suggesting very small doses of caffeine, equivalent to half a cup of coffee, are enough to induce a pharmacological effect.

1.6.1 Reversal of withdrawal or genuine cognitive enhancement

The possibility of caffeine's apparent effect occurring secondary to withdrawal reversal has been factored into several study designs producing mixed results. Childs and De Witt assessed the physiological, subjective, and behavioural effects of caffeine in 102 light caffeine users whom they report as non dependent (discussed below) and hence unable to experience withdrawal. Participants were tested with placebo, 50, 150, or 450 mg caffeine in random order during the four test sessions, separated by 72 hours. The results demonstrated a dose dependent improvement in the Visual Vigilance task, a measure of alerting attention. However, there was a decrease in backwards digit span with the 450mg dose suggesting an impairment of working memory or possibly executive function. 150 mg is overall the best dose as it produced improvements in alerting attention without the negative effects on working memory.

Following overnight withdrawal caffeine improved cognitive performance on alerting and executive attention tasks and a working memory task irrespective of habitual caffeine intake (Haskell et al., 2005). Haskell et al concluded that acute caffeine ingestion of greater than 75mg is large enough to produce cognitive benefits beyond that of withdrawal alleviation. Of the 48

participants enrolled in the trial, only 30 were available for full analysis (37.5% drop out / exclusion rate) and the reason for this was not adequately disclosed which seriously undermines the validity of these findings. The most likely adverse effect of trial participation would be caffeine withdrawal symptoms; it may be those most affected self terminated further participation, making the remaining participants a self selecting group not generalizable to the rest of the population.

Comparing regular consumers who abstained from caffeine overnight with non-consumers has been proposed by Hewlett and Smith to discriminate whether caffeine withdrawal influences cognitive performance (Hewlett and Smith, 2006). The premise was physiological caffeine withdrawal could be confirmed or refuted by this comparison as non-consumers, by definition could not experience caffeine withdrawal. Compared to baseline testing, caffeine ingestion after overnight abstention reduced (i.e. improved) choice reaction time in regular consumers with the effect on high consumers greater than low consumers but increased the choice reaction times in non-consumers. The explicit variation in alerting attention (represented by choice reaction time) according to caffeine consumption pattern should be interpreted as evidence for reversal of withdrawal. Yet the authors' claim "the present results showed no evidence for any detrimental effect of a period of caffeine withdrawal on cognitive performance". This statement cannot justifiably be made as the baseline in consumers was taken following a minimum of 7 hours caffeine abstinence putting the participant into withdrawal. If a baseline had been taken prior to withdrawal during a normal caffeinated state and then compared to testing following overnight withdrawal, this would allow for an accurate analysis of withdrawal effects to be made.

A more recent study by Rogers et al (Rogers et al., 2013) broadly replicated Hewlett and Smith's design and therefore the same criticisms apply. From their 369 participants randomly allocated placebo or 300mg caffeine (in two divided doses) following overnight withdrawal, they demonstrated an increase in motor speed but not in cognitive processing. Interestingly the

improvement in motor speed occurred to the same degree irrespective of habitual caffeine use and showed no decline from caffeine abstention. In low or high caffeine consumers caffeine had no significant effect on alerting attention tasks, however, participants who received placebo demonstrated performance deterioration, likely as a consequence of increasing withdrawal as testing was repeated at set time points throughout the day. In keeping with his original hypothesis Rogers' study supports no acute net benefit in efficiency of cognitive performance for high caffeine consumers.

The preload design was first utilised by Warburton which he proposed would counter possible effects of caffeine withdrawal (Warburton, 1995). By issuing all participants with 75mg caffeine 1 hour prior to attending the trial, he subsequently tested mood and cognition on 75mg and 150mg caffeine against placebo. Yeomans et al replicated and modified his study design using a variable caffeine preload of 0, 1 or 2 mg/kg caffeine followed by a test condition where all participants received 1mg/kg caffeine (Yeomans et al., 2002). Warburton found a positive effect of caffeine whereas Yeomans found no statistical difference. Yeomans demonstrated administering a preload of 1 or 2 mg/kg caffeine eliminated the positive effect of subsequent caffeine during the testing phase. This would be consistent with reversal of withdrawal effect. It is conceivable Warburton's study showed positive effects due to the preload caffeine dose being too small to completely reverse withdrawal (James, 1998). In conclusion the preload design is insufficient to characterise the effects of caffeine independent of its effects on withdrawal reversal.

Most caffeine studies only assess the alerting network with regard to attention but Brunye (Brunye et al., 2010) is the first group to systematically assess attention, modelling their paradigm to correspond to Posner's trinity of attentional networks. They tested 36 young adults with an average caffeine consumption of 42.5 mg/day using Posner's attentional network test. Following overnight withdrawal they received one of four variable doses between 0 and 400 mg, crossing over on subsequent sessions to receive each different dose type.

The most potent dose was 200 mg of caffeine, which improved performance on alerting and executive attention but slightly diminished orienting attention function. Given the abstention procedure, the question of withdrawal reversal is reasonable. They took pre-test salivary caffeine samples but did not formally check them to ensure abstention compliance. Furthermore they compared the practice day to the 0 mg day and found no statistical difference in performance, which they concluded as excluding withdrawal. This is possible although testing should always be better than practice unless (i) there is no practice effect in which case a practice day is unnecessary or (ii) another factor i.e. withdrawal, nullify any improvement due to practice.

This study is interesting because it clearly demonstrates the Yerkes-Dawson law applies to caffeine, with different attentional network domains being optimised by different doses. The study also proposes that dopaminergic stimulation rather than adenosine antagonism is the mechanism that underpins attentional enhancement not just in the alerting but also the executive domain. The rational for this assertion is based on the prefrontal cortex and thalamus, areas associated with alerting attention, being densely innervated by dopaminergic neurons. This study is worthy of replication but with a prolonged caffeine abstention to negate any argument of withdrawal reversal.

1.6.2 Low consumers labelled as non-consumers

Testing non-consumers or comparing them with consumers has been mooted as an approach to circumvent caffeine withdrawal reversal as a confounding factor. Whilst in principle this is plausible, the practicalities of identifying participants who do not consume the world's most frequently ingested psychostimulant is difficult. Non-consumers may represent a cohort inherently unresponsive or hypersensitive to caffeine rendering them unsuitable for investigation. The critical issue in labelling intermittent low consumers as non-consumers, is whether dependence or tolerance is possible at their level of caffeine intake. As described above, caffeine doses of 100mg a day (2 cups of instant coffee) can induce dependence and 25mg a day is enough to prevent significant withdrawal symptoms in heavy habitual caffeine users. Therefore it

would only be acceptable to categorise participants who consume less than 25mg of caffeine a day (or up to 175mg a week) as non-consumers.

Participants have systematically been labelled non-consumers despite consuming over 175mg of caffeine a week. Childs and de Witt recruited participants who consumed up to 300mg of caffeine per week, which is approximately equivalent to one cup of tea per day (Childs and de Wit, 2006). However, habitual use was quantified by "estimates of 50 mg per 12 oz. serving of caffeinated soft drinks, 60 mg per 8 oz. serving of tea, 100 mg per 8 oz. serving of coffee". These caffeine values, which are considered for an American population, appear twice as great as expected in a UK population. American products may be fortified with more caffeine than UK products or Childs and de Witt over estimated the caffeine content of foodstuffs, making their population less liable to withdrawal. Hence the positive effects demonstrated in their study could represent acute improvements in attention rather than withdrawal reversal.

Comparing habitual users to non-consumers should allow the direct effects of caffeine to be assessed against withdrawal reversal in the respective populations. Haskell et al investigated the acute cognitive and mood effects of 75mg and 150mg caffeine in 24 habitual users and 24 habitual non-users, after overnight withdrawal. Habitual non-users were defined as low caffeine users who did not consumer tea or coffee but were permitted caffeinated soft drinks and ingested less than 50mg of caffeine a day. Baseline salivary caffeine mean values were 0.50 μg/ml for consumers and 0.36 μg/ml for non-consumers; it is surprising that following overnight withdrawal, participants labelled as "non-consumers" have a caffeine level 70% the value of regular consumers but the authors failed to address this issue.

It has been argued a serum caffeine level <1 μg/ml would be consistent with 24 hours withdrawal (Smith et al., 1982, Jacobson et al., 1994), however, this is based on studies which have relied on participant honesty rather than direct observation of caffeine abstention in a controlled environment. In these

cohorts approximately 50% have no measurable caffeine levels after 24 hours, which would be expected given the half life of caffeine is 4 hours, allowing 6 half lives to pass i.e. serum levels should be 1.5% of peak levels that occur following ingestion. As a point of comparison, following ingestion of 6mg/kg of caffeine, peak serum concentrations are approximately 40 μg/ml (Skinner et al., 2014) so after 24 hours of abstinence expected levels would be 0.6 μg/ml. Typical habitual caffeine users consume approximately 3mg/kg caffeine (3-4 cups of coffee) over the course of a day, so expected levels after 24 hours would be 0.3 μg/ml. Levels higher than this raise the suspicion of non compliance with caffeine abstention. The only possible explanation for detectable serum caffeine levels despite abstention would be intra and inter individual variation in elimination producing an extended half life (Kalow et al., 1998, Balogh et al., 1992).

1.6.3 Caffeine can improve subjective performance without objective improvements

Interestingly low dose caffeine of 50 mg improved self reported alertness in the absence of actual objective improvement on neuropsychological tasks suggesting a dissociation between mood and cognitive effects (Childs and de Wit, 2006). Childs and de Witt did not explore the reason behind this phenomenon; it is conceivable this is a direct mood effect attributable to the dopamine reward system or a manifestation of withdrawal reversal, which their trial design inconclusively differentiated.

The studies described above do not clearly delineate between the observed effects of caffeine as a consequence of withdrawal alleviation and direct pharmacological effects. The diverse findings likely reflect methodological heterogeneity such as abstention procedures and differences in caffeine dose or administration, rendering it difficult to draw firm conclusion on the absolute merits of caffeine. The definitive way to differentiate between withdrawal reversal and direct effects is to withdraw participants completely from all caffeine for at least five days prior to testing and provide decaffeinated alternatives to aid compliance. If prolonged caffeine abstention is performed

correctly, one will expect a significant drop out rate due to the adverse side effects of withdrawal, as occurred in both Rogers 2005 (see below) and Haskell's studies. In fact this could be considered a marker of an adequate withdrawal procedure. Published review articles have consistently evaluated caffeine research with inadequate withdrawal procedures. It is therefore unclear if caffeine generates any beneficial attentional effects beyond the reversal of withdrawal.

1.7 Systematic review of studies using withdrawn participants

The majority of studies citing a beneficial effect of caffeine use a typical withdrawal period of 12-24 hours, which is categorically inadequate and puts participants into a state of withdrawal. It is impossible to discriminate between withdrawal reversal and additional cognitive enhancement with this type of study design. A focused review of caffeine studies with an adequate withdrawal period is warranted. There are no reviews which exclusively examine caffeine trials with an adequate withdrawal protocol. Caffeine withdrawal symptoms peak by 51 hours but range between 2 to 9 days, therefore studies incorporating a withdrawal period greater than 4 days (96 hours) should have the majority, if not all participants in a non dependent state when tested on caffeine. This review will therefore *only* assess studies with a withdrawal period of greater than or equal to 4 days.

A systematic literature review was conducted, according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (Moher et al., 2015). The following characteristics and inclusion criteria were used:

(i) studies on humans
(ii) the caffeine intake/dose was reported
(iii) papers were randomised controlled trials
(iv) the study was not exclusively a combined multi-intervention
(v) access to the full text paper was available
(vi) peer reviewed publication.

The following search strategy was used: MEDLINE database (PubMed) was searched for English language, peer reviewed human studies published between May 1953 and 2017, with the last check run on the 23rd of March 2017. The keyword search terms included:

1. caffein* OR tea OR coffee OR (energy drink) OR (red bull) OR redbull; 32,308 results
2. attenti* OR attention OR alert* OR alertness OR cognition; 454,533 results
3. randomi* AND trial*; 595,030 results
4. 1 AND 2 AND 3; 300 results. 63 relevant results

A further 18 studies were found through bibliographies and search engines such as Google scholar. No further articles studies were found after applying the same search strategy to PSYCHinfo and the Cochrane Library. Of the 81 studies reviewed only 4 studies used a withdrawal period of at least 4 days, these are summarised individually below. Due to the heterogeneity of participant group, caffeine dose, withdrawal duration and attentional outcome measure, a meta-analysis was not feasible.

1.7.1 Research paper 1: Acute effects of caffeine on attention: a comparison of non-consumers and withdrawn consumers (Smith et al., 2013)

The acute effects of caffeine in non-consumers were compared with withdrawn consumers following a 7-day caffeine washout period. Participants were requested to abstain from all caffeinated drinks for the duration of the trial and the effects of withdrawal were examined by carrying out performance testing on Day 2 of abstention. On day 8 both non-consumers and consumers were randomly assigned to caffeine or placebo conditions. The authors conclude short term withdrawal produced a subjective but not an objective decline in performance in regular consumers.

Both withdrawn consumers and non-consumers showed improved simple and choice reaction times 30 minutes following caffeine ingestion on day 8. This appears to be a robust finding in non-consumers at least and points towards caffeine producing an objective improvement in alerting attention beyond that of

practice effect. A criticism of their results analysis is the lack of recognition of caffeine's ability to improve motor speed (Bazzucchi et al., 2011, Rogers et al., 2013) as opposed to cognitive processing speed (cognitive reaction time = choice reaction time minus simple reaction time). In this particular case whilst cognitive reaction time was not formally calculated, a decrease in error rate is sufficient to demonstrate a positive cognitive effect. The caffeine dose of 2mg/kg was similar to the most effective dose of 150mg in Childs and de Witt's study which produced similar effect findings whereas Rogers used 1.2mg/kg which may have been too low to produce a measurable neuropsychological effect.

Saliva samples were taken on Days 2, 4 and 8. Surprisingly caffeine levels increased from day 2 to days 4 and 8 in the withdrawn consumer group suggesting caffeine ingestion was still taking place. The authors suggest this was in low doses from other dietary sources such as chocolate, however, with a level of 0.18 μg/mL and a standard deviation of 0.14, suggests significant caffeine intake in at least some of the participants, irrespective of the source. It may be the assay is inaccurate below 0.5 μg/mL as suggested by Rogers in his study (Rogers et al., 2005), if this is the case, the utility of caffeine levels is to exclude high caffeine intake i.e. >200mg/day but is redundant to accurately confirm or exclude low caffeine intake unless the value is zero. The critical issue is at what level does caffeine intake induce dependence and hence produce withdrawal on abstention. In reality this is likely to vary between individuals due to metabolising enzyme ADORA2A polymorphism.

1.7.2 Research paper 2: Effects of caffeine and caffeine withdrawal on mood and cognitive performance degraded by sleep restriction (Rogers et al., 2005)

The effect of acute caffeine versus placebo was assessed on sleep deprived, habitual caffeine consumers who were either withdrawn overnight or unknowingly undertook a 3 week withdrawal, on simple reaction time, choice reaction time and the test of variables of attention. The authors intentionally avoided comparing caffeine consumers with non-caffeine consumers as they are self selecting groups and therefore variation in response could be accounted for by pre-existing differences. The results demonstrated the baseline assessments

of overnight withdrawn participants were worse than those on long term withdrawal and caffeine only improved the cognitive performance of overnight withdrawn participants; supporting the withdrawal reversal hypothesis. The fully withdrawn participants did not demonstrate any improvement on cognitive performance following caffeine. Rogers therefore concluded caffeine does not have any net beneficial effects on cognition in young healthy individuals; the study population were university students aged 20 to 34 tears old. The difficultly of executing this or similar experimental paradigms is highlighted by the failure of one third of participants to comply with caffeine restriction, evaluated by salivary caffeine levels prior to testing.

1.7.3 Research paper 3: Effect of chronic caffeine intake on choice reaction time, mood, and visual vigilance (Judelson et al., 2005)

The cognitive effect of chronic caffeine intake was studied by Judelson et al. All participants ingested 3mg/kg caffeine for the first 5 days following which they were randomly allocated to either placebo, 3mg/kg or 6mg/kg of caffeine, split into 2 daily doses, for the following 5 days. The study aim was to assess whether tolerance developed to the acute cognitive effects of caffeine rather than to assess fully withdrawn participants against acute caffeine ingestion. A four choice reaction time and a scanning vigilance task showed no statistical difference between any of the groups. The authors correctly conclude that either there are few measurable cognitive effects of chronic caffeine use or complete tolerance to any positive effects of caffeine develop within 5 days. It is rare for negative studies to be published and among the 81 randomised controlled trials reviewed this has a robust paradigm. An area for concern is the limited neuropsychology tested - four choice reaction time test and scanning visual vigilance test. As discussed above the choice reaction time on itself does not differentiate changes in motor speed from cognitive processing speed. There is also no testing of executive attention and therefore this study is prone to type 2 error.

1.7.4 Research paper 4: Caffeine improves reaction time, vigilance and logical reasoning during extended periods with restricted opportunities for sleep (Kamimori et al., 2015)

Sleep deprived special forces soldiers were used to test the effect of repeated doses of caffeine, totalling 800mg/day versus placebo with caffeine abstention over 4 days. On the psychomotor vigilance test, which requires participants to press a designated computer key as soon as possible after a visual cue appears on a computer screen, those consuming caffeine were significantly faster than placebo after 2,3 and 4 days of caffeine abstention. Whilst testing was performed throughout the day, statistical significance was only obtained during the night hours and lost during daytime testing. This is an interesting finding as attention and cognitive function mirrors circadian rhythm (MONK et al., 1997) and this result does not clearly differentiate wakefulness effects from true attention enhancement. It may be caffeine does not affect attention in cognitively normal individuals but does have an effect when attention is impaired such as by sleep deprivation.

Study	Subjects	Study Design	Caffeine intervention	Caffeine Withdrawal Duration	Attentional Outcome Measures	Main findings	Study Weaknesses
Acute effects of caffeine on attention: a comparison of non-consumers and withdrawn consumers (Smith et al., 2013)	35 habitual consumers >100mg caffeine/day and 35 non consumers; age 18-53 year, mean 22.8 years	Non-consumers were compared with withdrawn consumers over a 7-day washout period. Effects of withdrawal on the attention tasks were examined by carrying out performance testing on Day 2 of withdrawal. On day 8 a double-blind placebo-controlled caffeine challenge was carried out, with non-consumers and consumers being randomly assigned to caffeine or placebo conditions.	2 mg/kg single dose in decaffeinated coffee or tea.	7 days	Simple reaction time, focused attention task, categoric search task, repeated digits detection task	Following 7 days withdrawal, ingestion of caffeine was associated with faster simple reaction time, fewer long responses, greater detection of targets in the cognitive vigilance task and faster encoding of new information in both non-consumers and withdrawn consumers, with no difference in effect size between them.	Habitual consumers after 24 hours of withdrawal performed worse than non-consumers on simple reaction time, number of targets detected and speed of encoding but there was not enough power to elicit a statistical significance.
Effects of caffeine and caffeine withdrawal on mood and cognitive performance degraded by sleep restriction (Rogers et al., 2005)	48 moderate to high caffeine consumers; 20-34 years	23 participants were provided with decaffeinated tea/coffee and 25participants with regular tea/coffee in the 3 weeks prior to testing; on the night before testing, participants' sleep was restricted to 5 hours; a double blind placebo controlled caffeine challenge with cross-over on repeated testing 2 days later.	1.2 mg/kg single dose in blackcurrant squash	21 days for 50% of participants, overnight for the other 50%	Simple reaction time, choice reaction time, the test of variables of attention.	Overnight caffeine withdrawal was associated with negative cognitive effects. Caffeine ingestion had no positive cognitive effects.	Of the participants who underwent 21 days of caffeine abstention, 50% were given caffeine and then re-tested on placebo 2 days later, meaning technically they could be in withdrawal. The authors felt the modest testing dose of caffeine would not affect their status as long term withdrawn.
Effect of chronic caffeine intake on choice reaction time, mood, and visual vigilance (Judelson et al., 2005)	60 habitual consumers, ages not declared	Participants were divided into three randomized, balanced, blinded groups of 20 subjects; on days 1–6 all participants consumed 3 mg/kg of caffeine; day 7-12 groups were allocated to either placebo, 3mg/kg or 6mg/kg caffeine.	Placebo, 3mg/kg or 6mg/kg per day split in 2 divided dose capsules.	5 days for 33.3% of participants	Four choice reaction time test, scanning visual vigilance test.	No difference between placebo and chronic (5 day) caffeinated states on tests of attention.	Underpowered study.
Caffeine improves reaction time, vigilance and logical reasoning during extended periods with restricted opportunities for sleep (Kamimori et al., 2015)	20 Special Forces soldiers aged 28.6 ± 4.7 years	50% receiving caffeine and 50% placebo during a 4 day period of caffeine abstention with sleep restricted to 4 hours a night	800mg per day in 4 divided doses of chewing gum, for 4 days	4 days for 50% of participants, other participants not withdrawn	Psychomotor vigilance test	Sleep deprived participants receiving caffeine were significantly better on days 3,4 and 5 when tested overnight and early morning but not when tested during the day.	The positive effects of caffeine being limited to nocturnal testing were not adequately explained.

Table 1.2 Summary of the systematic review

1.7.5 Systematic review conclusion

From the randomised controlled trials which employed a withdrawal period of at least 4 days, two themes have emerged. Firstly, *in cognitively normal* individuals, neither acute nor chronic caffeine ingestion clearly improves attention. In accordance with the Yerkes-Dodson law, which asserts an optimal level of attention cannot be enhanced i.e. healthy individuals are already functioning at optimum attention which cannot be improved, however, cognitive stimulants could cause a deterioration in performance. The second theme, is in sleep deprived (and by extension temporarily cognitively impaired) individuals, caffeine has a reproducible effect in improving attention. As discussed in section 1.3 Caffeine pharmacology, adenosine levels increase with sleep deprivation, especially in the basal forebrain and inhibit the activity of wakefulness promoting cholinergic and aminergic neurons. Caffeine, through adenosine receptor inhibition, will not only delay sleep but promote wakefulness and increased attention through basal forebrain cholinergic enhancement (Porkka-Heiskanen and Kalinchuk, 2011).

This is exciting as caffeine studies are almost exclusively tested in healthy populations, yet they have the potential to act as cheap, safe, cognitive enhancers in neurological conditions characterised by attentional deficits.

There are no randomised trials assessing the effect of acute caffeine on attention in elderly or cognitively impaired participants and this is an area worthy of exploration. Studies examining the cognitive enhancement properties of caffeine are typically performed by experimental psychologists using a young adult population. When trying to extrapolate these findings to healthy elderly people they are limited by differences in drug metabolism by the liver or absorption due to decreased gastro-intestinal transit. This is well established for drugs such as benzodiazepines where smaller doses are recommended with advancing age (Swift et al., 1985b). It has been stipulated that elderly participants are less prone to the subjective mood enhancement of caffeine but more sensitive to objective psychomotor improvement (Swift and Tiplady, 1988b). Serial caffeine

concentrations and psychomotor performance following caffeine ingestion in healthy elderly participants has been examined, however, the time at which peak plasma concentration develop were not reported (Bryant et al.). However, it was noted that doses of 116mg produced a superior beneficial psychomotor effect than higher doses of 231mg. Since the majority of studies use caffeine doses of 150-400mg, they may be overdosing their participants and missing positive effects only appreciable following administration of lower doses.

1.8 Aims

The efficacy of caffeine as an attentional enhancer has not been clearly elucidated beyond reversal of withdrawal. In cognitively normal, young participants there is no clear effect. However, in individuals cognitively impaired secondary to sleep deprivation, it has positive effects. It is possible these positive effects are transferable to cognitively impaired individuals associated with neurological disease rather than sleep deprivation.

The aim of this thesis is to determine if caffeine is an attentional enhancer independent of its effects on withdrawal reversal. This will be assessed in healthy elderly and cognitively impaired patient populations. Further, I will explore which subtype of attention is enhanced by caffeine in each group.

1.9 Structure of the thesis

The current chapter has appraised the literature concerning the attention enhancing properties of caffeine. Chapter 2 describes the general methods used throughout the four data chapters including descriptions of experiments used and the rationale behind statistical analysis. Chapter 3 assesses the effect of caffeine in dementia with Lewy bodies and aged matched health individuals using version 1 of

the protocol. Chapter 4 assesses the effect of caffeine on a large cohort of healthy elderly participants. Chapter 5 assesses the effect of caffeine on participants with Parkinson's disease. Chapter 6 assesses the effect of caffeine on participants with multiple sclerosis. Chapter 7 provides a general discussion of all the data contained within the thesis including conclusions drawn from them and future directions for research.

Chapter 2

General Methods

This chapter details the general methods that were used throughout this thesis. Each chapter describes the same experimental paradigms but with different parameters which are specified individually. The majority of experimental testing was carried out by the author, though occasionally some healthy participants were tested by other members of the laboratory (MSc students Thomas Davies, Scott Ankrett and BSc student Greg Munro). The author formulated experimental design and undertook all of the analysis.

Ethics approval was granted by the NRES Committee South West - Exeter and all participants gave written consent in accordance with the World Medical Associations revised Declaration of Helsinki (2013).

2.1 Participants

Healthy older participants were recruited in one of two ways. Some participants were the spouses of the Parkinson's disease (PD) or dementia with Lewy bodies (DLB) patients that came in for testing, and these were tested at the same time as their spouses in a separate room by a separate experimenter. The second way was via the BRACE (**Br**istol **A**lzheimer's and **C**are of the **E**lderly) Healthy Volunteer Database. This database is maintained by the ReMemBr (**Re**search into **Me**mory and the **Br**ain) Group and contains contact details for healthy volunteers who wish to take part in research. Potentially eligible participants on the database were initially contacted by post.

2.1.1 The inclusion criteria for healthy older participants were:

- vision sufficient to carry out tasks
- an adequate level of communication in written and verbal English
- independently mobile

2.1.2 The exclusion criteria for healthy participants were:

- any concomitant serious neurological or non neurological illnesses likely to interfere with cognitive or physical performance
- any reported cognitive problems
- signs of cognitive impairment (e.g. Montreal Cognitive Assessment lower than 26)
- inability to consent to research, in keeping with the Mental Capacity Act 2005

For patient studies, PD, DLB and multiple sclerosis (MS) patients were first identified through clinical research databases kept by research nurses at North Bristol NHS Trust. Once this had been exhausted, specialist and general neurology clinics were screened for potential participants as long as they fulfilled inclusion and exclusion criteria for the study. All patients were vetted by the clinician involved in this study (the author) and the patient's own consultant neurologist prior to being deemed eligible to be contacted regarding the study.

2.1.3 The inclusion criteria for neurological participants for all experiments were:

- an established clinical diagnosis of PD, DLB or MS
- subjective or objective cognitive impairment
- if taking cognitive enhancers i.e. cholinesterase inhibitors or memantine, stable on medication for 3 months or more
- normal visual acuity or normal corrected vision

- an adequate level of communication in written and verbal English
- independently mobile

2.1.4 The exclusion criteria for neurological participants were:

- any other concomitant serious neurological or non neurological illnesses likely to interfere with cognitive or physical performance
- inability to consent to research, in keeping with the Mental Capacity Act 2005
- loss of capacity to consent to research during the trial

For both healthy and patient recruitment, participant information sheets and reply slips were posted out. Upon return of the reply slip (which included the participant's email or phone number), the participant was contacted and if they wished to take part, visit dates were agreed. Non responders were followed up after two weeks with a courtesy phone call to ensure they had received the information and to answer any questions.

2.1.5 Withdrawal criteria for all participants

- Development of any of the exclusion criteria during the trial
- Losing the capacity to consent to treatment or testing during the trial
- Inability to tolerate caffeine withdrawal symptoms or maintain caffeine abstinence
- Change to any medication except non opiate analgesia and anti hypertensives

2.2 Caffeine withdrawal procedure

Participants both healthy and those with cognitive impairment were initially tested whilst on their habitual caffeine intake and subsequently undertook a standardised procedure for caffeine withdrawal as follows.

Participants attended for baseline testing on day 1 without any dietary caffeine restriction. Following testing they were given a supply of either decaffeinated coffee or decaffeinated tea to cover the trial duration (as per their consumption preference) and requested not to ingest caffeine containing foods such as tea, coffee, chocolate etc. (a comprehensive list was issued) for the remainder of the trial (9 days) but could freely consume the decaffeinated tea/coffee supplied to them. On day seven (i.e. 1 week free from caffeine) participants repeated testing to assess for effects of caffeine withdrawal on attention and allow task familiarisation so the effect of learning on subsequent performance was minimised as a factor. On day eight participants received either caffeinated or decaffeinated coffee and 60 minutes following completion of consumption commenced testing. In the interim, participants waited in a quiet waiting room or the testing room with books and magazines for interest if desired. On day nine the participants received the alternative type of coffee (caffeinated or decaffeinated whichever not already had) and began testing 60 minutes following consumption. Testing was performed at the same time on all days to prevent changes in cortisol levels confounding data analysis.

If receiving caffeine on day 8 and placebo on day 9, it was assumed this single exposure to a modest dose of caffeine would not significantly affect their status as fully caffeine withdrawn. It has been previously demonstrated that when habitual caffeine consumers were made abstinent for seven days, then given 300mg caffeine/day for just one day, they displayed no subsequent effects of withdrawal. However, if following abstinence they maintained 300mg caffeine/day for three or more days, then significant withdrawal symptoms developed on stopping this maintenance dose (Evans and Griffiths, 1999).
The alternative procedure of withdrawing them again for a further week, increasing total testing duration to 15 days was felt to be unnecessarily demanding on the participant, a sentiment echoed in another high quality trial (Rogers et al., 2005).

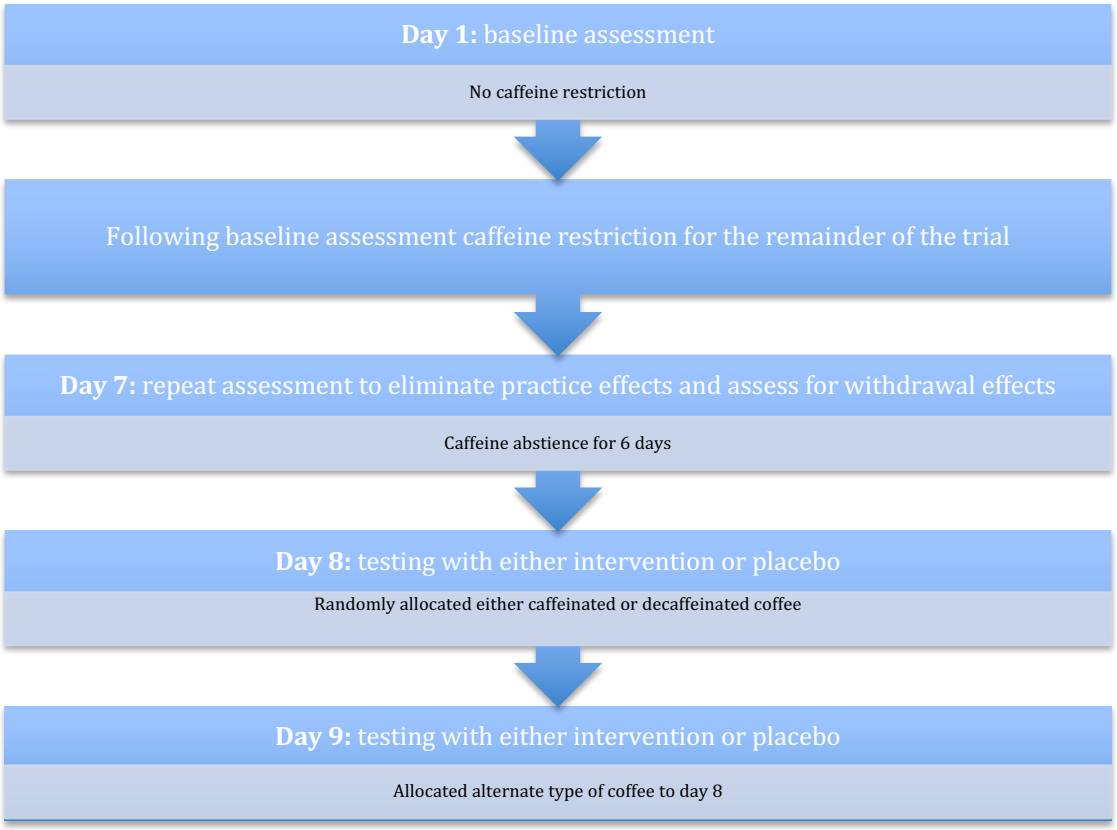

Figure 2.1 Caffeine withdrawal procedure

2.2.1 Effect of caffeine withdrawal

Following caffeine abstinence in habitual consumers, withdrawal symptoms predictably commence after 12 to 24 hours, with symptom severity maximal between 20 to 51 hours, and duration of withdrawal characteristically extending between 48 hours up to nine days. Experimental data suggests the most common withdrawal symptom is headache, affecting approximately half of all abstainers. Other withdrawal symptoms can affect mood (irritability, depression, malaise), cognition (decreased alertness and concentration, drowsiness, fogginess) and physical wellbeing (flu-like symptoms, muscle aches, nausea, vomiting). Symptom severity is enough to cause functional impairment or distress in 13% of experimental studies, with the incidence being lower in survey data (Juliano and Griffiths, 2004).

Caffeine withdrawal symptoms, by definition should only develop following acute abstinence, intensify in severity and subsequently diminish to complete resolution with continued abstinence. The sequential evolution of these phases are essential to differentiate withdrawal effects from those attributable to the loss of caffeine's therapeutic effect (Juliano and Griffiths, 2004).

There is no published evidence to suggest a graded caffeine withdrawal will produce fewer or less severe side effects of withdrawal than abrupt cessation. Caffeine studies have been performed for over 100 years and so caffeine withdrawal is known not to be harmful although some of symptoms people experience can be distressing.

2.3 Questionnaires

2.3.1 Caffeine intake

A caffeine questionnaire assessed baseline caffeine intake on the day the trial began. Caffeine consumables were divided into seven general categories: coffee (e.g., specialty coffee drinks, iced coffee, brewed, instant, and decaffeinated coffee), tea (e.g., green tea, white tea and other varieties, iced tea), caffeinated soft drinks, chocolate drinks (including milk and cocoa), chocolate bars, medications and others.

During the trial participants were required to complete and return a daily caffeine consumption questionnaire to ensure adherence to caffeine abstinence.

CAFFEINE CONSUMPTION QUESTIONNAIRE
- Caffeine trial

Please answer the following questions as completely and honestly as you can. This information is STRICTLY CONFIDENTIAL – do not write your name anywhere on this page. Thank you for your cooperation.

Log all items that you consumed yesterday.
For drinks **list the number of cups** you have during each time period and the type/brand e.g. for coffee it could be instant, ground, espresso, percolated etc.

FOOD/DRINK Type/Brand	MORNING 0600-1200	AFTERNOON 1200-1800	EVENING 1800-2400	NIGHT 0000-0600
Coffee				
Tea				
Hot Chocolate				
Cocoa				
Fizzy Drinks				
Other drinks				
Chocolate bar				
Over the counter medications				
Other				

2.3.2 Sleep questionnaire

This questionnaire recorded symptoms of daytime somnolence and sleeping habits for the duration of the trial. If the patient had a sleeping partner there are questions the sleeping partner can assist the participant in completing to assess for REM sleep behaviour disorder.

SLEEP QUESTIONNAIRE
- survey

DAY: MON/TUES/WED/THURS/FRI/SAT/SUN
(delete as appropriate)

Please answer the following questions as completely and honestly as you can. This information is STRICTLY CONFIDENTIAL. Record information for the day stated above and fill out 1 sheet for each day of the week.

PLEASE ANSWER THIS BOX OF QUESTIONS BEFORE GOING TO BED

QUESTION FOR PARTICIPANT BEFORE THEY GO TO BED	ANSWER
How many naps did you take during the day?	
Did you fall asleep: Sitting and reading?	(Circle answer) Yes / No
Watching TV?	Yes / No
When sitting and talking to someone?	Yes / No
When sitting after lunch?	Yes / No
In total how many hours did you nap for during the day?	

PLEASE ANSWER THIS BOX OF QUESTIONS THE MORNING AFTER YOU ANSWER THE QUESTIONS ABOVE

QUESTION FOR PARTICIPANT WHEN THEY WAKE UP IN THE MORNING	ANSWER
What time did you go to sleep?	
What time did you wake up?	
How many times did you wake up in the night?	
What causes you to wake in the night? e.g. to go to the toilet, woke up with pain etc.	
Approximately how long did you stay awake during the night in total?	
Did you feel refreshed when you woke up this morning?	Yes / No

IF YOU SHARE A BED WITH SOMEONE WHO CAN HELP WITH THE ANSWERS, PLEASE COMPLETE THIS BOX OF QUESTIONS

QUESTIONS WITH HELP FROM A BED PARTNER IF AVAILABLE	**ANSWER**
Did they say you snored in your sleep last night?	Yes / No
Did they say you had pauses in your breathing last night?	Yes / No
Did they say you screamed/shouted in your sleep last night?	Yes / No

2.4 Task battery

The task battery consisted of:

i. The Montreal Cognitive Assessment (MoCA)
ii. Digit span
iii. Simple reaction time
iv. Choice reaction time
v. The rapid serial visual presentation (RSVP) paradigm
vi. Stroop task
vii. Walking while talking test (WWT)

The battery was performed in the same order on each visit.

For computerised paradigms (iii-vi), Presentation software (Version 18.0 www.neurobs.com) was run on a 15 inch Toshiba laptop running 32-bit Windows 7 pro or a 15 inch Dell laptop with 64-bit Windows 7 pro. A Cedrus RB-844 response box was used to record participant responses, as this allowed greater precision in reaction time measurements than laptop keyboards and was easier for participants with motor difficulties.

2.4.1 Montreal Cognitive Assessment

Administration

At baseline testing, a Montreal Cognitive Assessment (MoCA) version 7.3 was performed. This was only performed at the baseline assessment on day 1, as part of the screening inclusion criteria in healthy participants and allowed an approximate gauge of cognitive impairment in participants with neurological disease.

Interpretation

This is a validated 30 point pen and paper cognitive test, lasting 10 minutes, used in clinical practice to screen for mild cognitive impairment and dementia (Nasreddine et al., 2005). The original validation study suggested a score of ≥26

would differentiate normal elderly (the sample mean was 75 years) from cognitively impaired. Over the past 10 years the validity of the cut off score has been debated with some studies finding presumed cognitively normal, elderly patients scoring anywhere between 19/30 and 30/30 (Gluhm et al., 2013). There is now consensus that is it more accurate to apply a normative approach to a cut off score based on age and years of education (Malek-Ahmadi et al., 2015).

Age	**Years of education**		
	≤12 Years	13–15 Years	≥16 Years
70-79	25.25 (4.11)	27.78 (2.24)	27.59 (2.04)
80-89	23.47 (2.97)	25.08 (3.13)	25.82 (2.75)
90-99	23.00 (2.63)	23.35 (3.43)	24.61 (2.59)

Table 2.1 Normative mean MoCA stratified by age and education (standard deviation in bracket) Adapted from Malek-Ahmadi et al 2015.

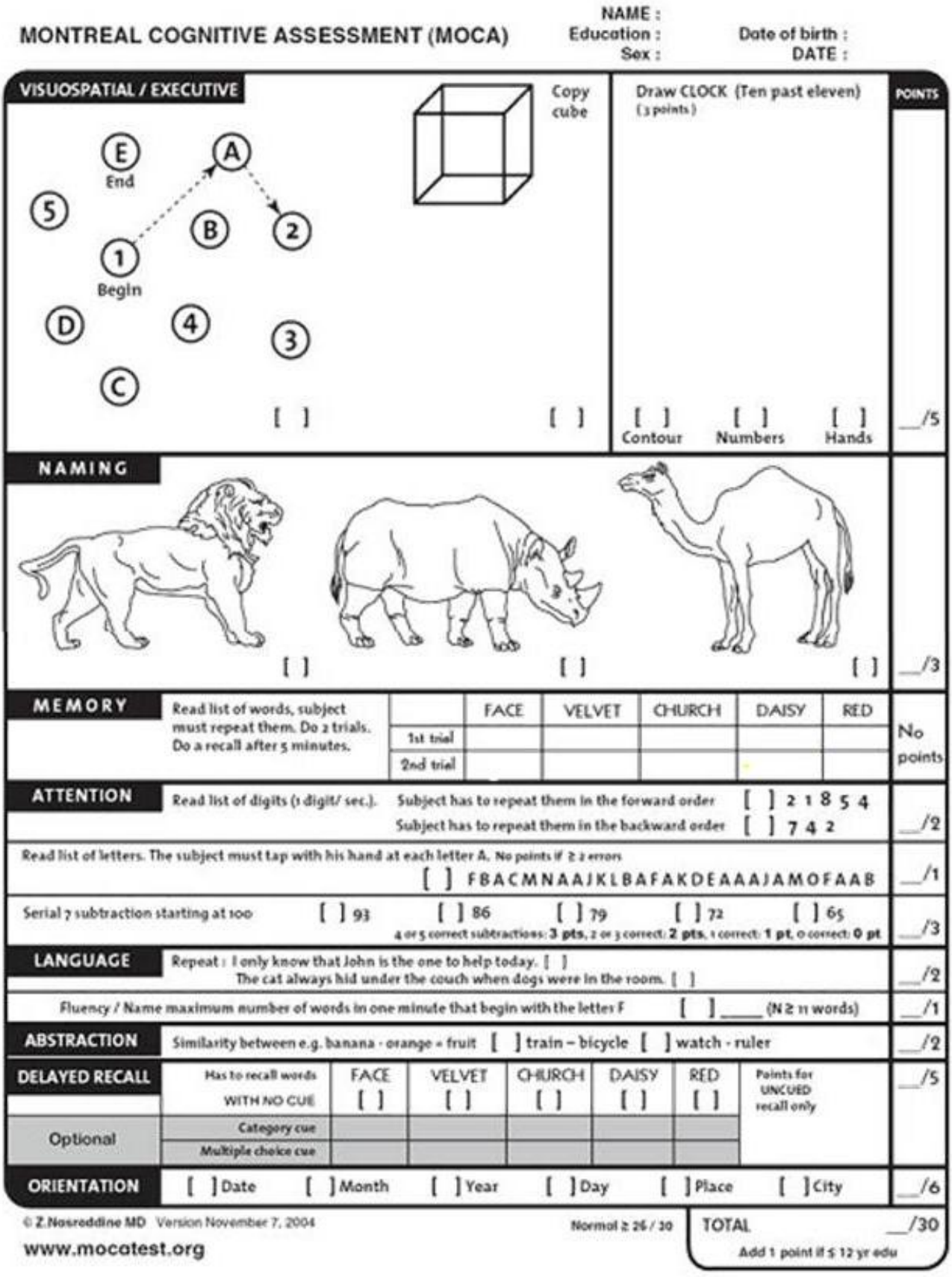

Figure 2.2 Montreal Cognitive Assessment (MoCA) (Nasreddine et al., 2005)

2.4.2 Digit span

Administration

Wechsler Adult Intelligence Scale fourth edition (WAIS-IV) digit span (Wechsler, 2008) was used as a test of attention in the context of working memory. Participants are audibly presented with a series of digits at 1 digit per second. The first testing block required participants to recite the digit span forwards and the second testing block required the digit span to be recited backwards. There were 2 trials per digit span length, starting at 2 digits long, with each consecutive digit span length increasing by 1 digit to a maximum of 9 digits forward and 8 digits backwards. The test was terminated if a participant failed to correctly recall both trials for a given digit span length. The dependent variable was the sum of digits forward and digits backwards, termed the reliable digit span.

Interpretation

Within working memory, forward digit span is predominantly a measure of attention efficacy whilst backward digit span is in addition a measure of transformation of information (Reynolds, 1997). Conventionally a reliable digit span is the measure of interest, which combines forward and backward digit span scores together. Reynolds et al have argued that forward and backward digit assess different aspects of working memory and therefore should be assessed separately. 2,200 American examinees were used to standardize and normalize the WAIS-IV across 13 age groups: 16–17, 18–19, 20–24, 25–29, 30–34, 35–44, 45–54, 55–64, 65–69, 70–74, 75–79, 80–84, and 85–90. Age groups of interest are tabulated below.

Age	Forward Digit Span	FDS sd	Backward Digit Span	BDS sd	Reliable Digit Span
25-29	11.0	2.3	9.0	2.5	20.0
30-34	11.0	2.5	9.0	2.8	20.0
35-44	11.0	2.5	9.0	2.8	20.0
45-54	10.0	2.5	9.0	2.5	19.0
55-64	10.0	2.8	8.0	2.5	18.0
65-69	10.0	2.5	8.0	2.5	18.0
70-74	10.0	2.5	8.0	2.3	18.0
75-79	10.0	2.5	8.0	2.0	18.0
80-84	9.5	2.3	7.0	2.0	16.5
85-89	9.0	2.0	6.5	1.8	15.5

Table 2.2 Normative mean digit span stratified by age (sd = standard deviation) (Wechsler, 2008)

2.4.3 Simple reaction time

Administration

Simple reaction time (SRT) – there is a single response to a single stimulus. Each time a 'red square' (2cm x 2cm) was presented in the centre of a computer screen the participant was required to press the corresponding 'red' coloured button on a free standing response pad as quickly and accurately as possible There was a variable fore period prior to stimulus onset of between 1500-3500 ms and stimuli were displayed for 2000 ms. The task comprised 10 practice trials and 100 test trials and responses between 100ms and 5000ms were recorded. The dependent variable was mean reaction time, measured from the onset of the stimulus until the participant's response on the response pad.

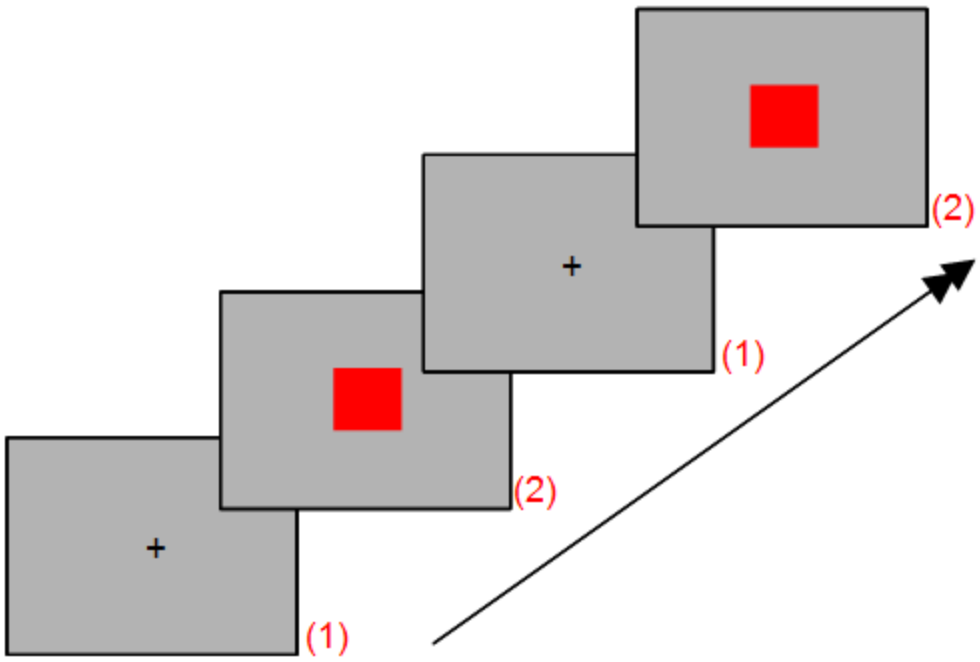

Figure 2.3 Simple reaction time sequence (1) Fixation target. (2) Repetitive stimulus requiring the same participant response on every occasion (Systems, 2018).

Interpretation

Simple reaction time measures the speed of mental processing on a singular task plus the time it takes to for the sensory input to reach the brain and for the motor response to be elicited. This can be considered a baseline reaction time due to the absence of competing or conflicting mental processes, such as when performing the choice reaction time (competing) or Stroop task (conflicting). A minimum simple reaction time is taken to be 200 ms although a century ago it was slightly faster at 190 ms, the reasons for which are unclear (IRWIN, 2010).

2.4.4 Choice reaction time

Administration

Choice reaction time (CRT) – there are two responses to two stimuli. Each time a 'red square' or 'blue square' was presented in the centre of the screen the participant was required to press the corresponding 'red' or 'blue' coloured button

on the free standing response pad as quickly and accurately as possible. There was a variable fore period prior to stimulus onset of between 1500-3500 ms and stimuli were displayed for 2000 ms. The task comprised 10 practice trials followed by 100 test trials and responses between 100ms and 5000ms were recorded. The dependent variable was mean reaction time, measured from the onset of the stimulus until the participant's response on the response pad. Cognitive reaction time was calculated by subtracting simple reaction time from choice reaction time.

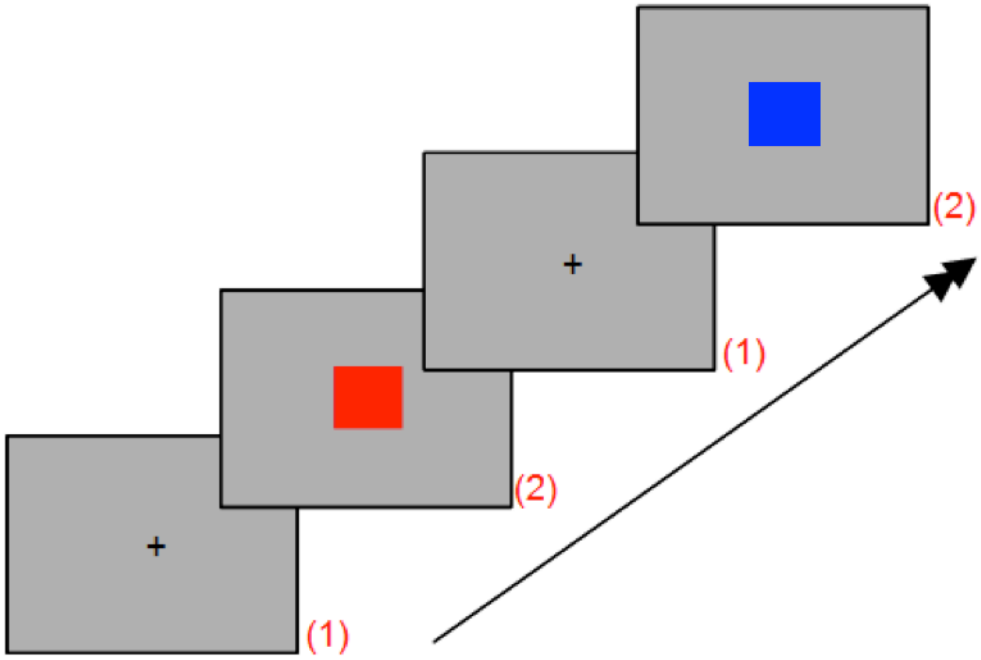

Figure 2.4 Choice reaction time sequence (1) Fixation target. (2) Variable stimulus requiring a specific participant response on every occasion (Systems, 2018).

Interpretation

If the simplest visual attention task requires a reaction time of 200 ms, by changing the complexity of the task, the increase in reaction time can be attributed to the mental processing. By subtracting the simple reaction time from the choice reaction time, the sensory and motor components of the task are negated and the

remaining value denotes the time required for the cognitive process, in this case, alerting attention.

2.4.5 Rapid serial visual presentation

Administration

Rapid serial visual presentation (RSVP) paradigm provides a framework to examine visual selective attention and its temporal dynamics. RSVP involves presenting a stream of randomly chosen letters presented rapidly in succession, at the centre of the screen. Each letter was presented for 131 ms with an inter stimulus interval of 49 ms equating to a presentation rate of 5.6 letters per second in keeping with recently published paradigms (Husain et al., 1997). Each RSVP stream was 25 letters long. All letters were black except the target letter (T1), which was red. The background throughout the sequence was a uniform grey. Each trial began with a black fixation cross lasting 500 ms. Prior to T1 the number of letters presented randomly varied between 7 and 15. T1 could be any letter except for "X". The second target letter (T2) was a black "X", randomly present in only 50% of trials. The T2 (letter X) was never presented before T1 (red letter) and no letter appeared twice within a single RSVP stream.

In the control block (single target trials) participants were requested to report the presence or absence of T2 only whereas in the testing block (dual target trials) participants were requested to identify T1 (by typing in the letter using the keyboard) followed by reporting the presence of absence of T2.

In Version 1 T2 onset could occur 360 ms, 720 ms, 1080 ms, 1440 ms or 1800 ms after T1. Reports of both targets were requested after the stimulus stream terminated. T2 was presented 3 times as each T2 time intervals, yielding a total of 15 T2 present and 15 T2 absent dual target trials. Participants completed 5 practice trials before each testing block.

In Version 2 T2 onset could occur after 180 ms, 360 ms, 540 ms, 720 ms, 900 ms, 1080 ms or 1260 ms. Reports of both targets were requested after the stimulus stream terminated. T2 was presented 3 times as each T2 time intervals, yielding a total of 21 T2 present and 21 T2 absent dual target trials. Participants completed 5 practice trials before each testing block.

The reasons for the 2 versions are discussed below (2.5.3 Motivation for protocol amendment).

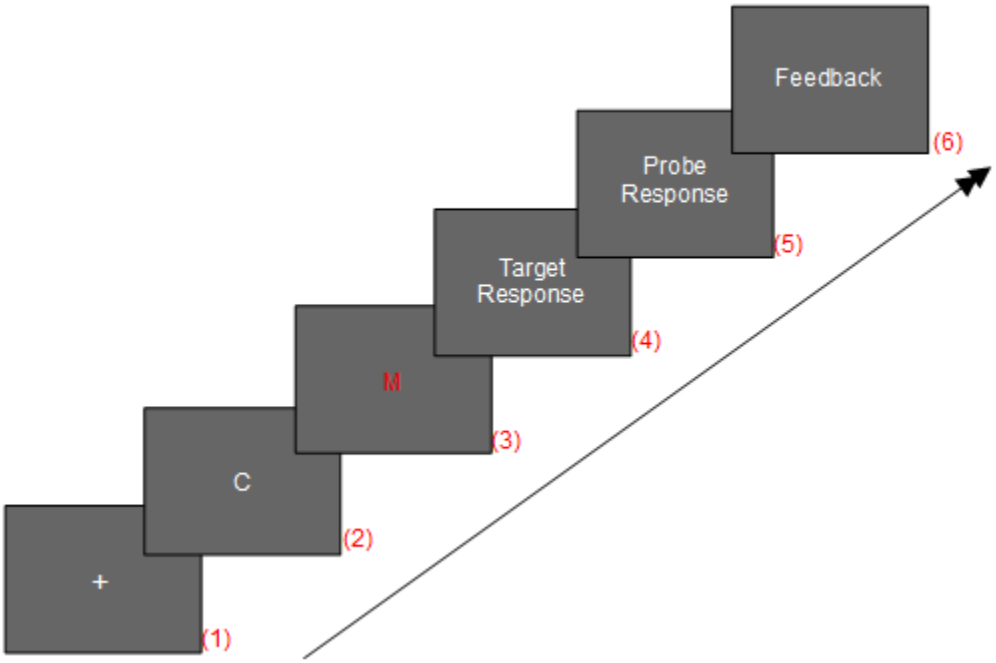

Figure 2.5 A visual representation of the RSVP trial sequence. (1) Fixation point to prime participant. (2) Letter sequence prior to T1. (3) Target (T1) letter in red followed by a further sequence of letters, in which X was present in 50% of trials. (4) Participant inputs the red target letter on the keyboard. (5) Participants inputs whether the letter X (T2) was present. (6) Participant advised whether X correctly identified (Systems, 2018).

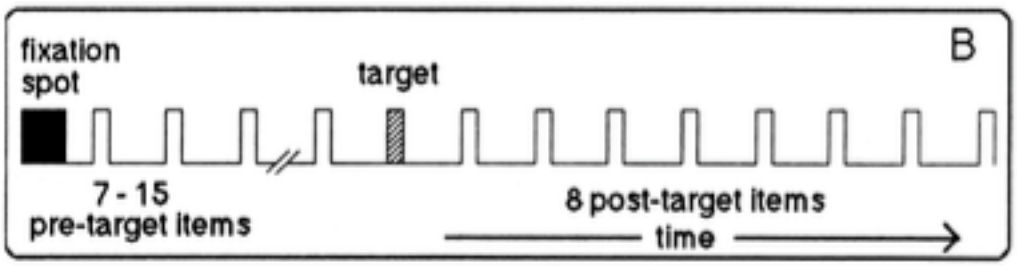

Figure 2.6 Pictorial representation of the RSVP paradigm (Raymond et al., 1992)

Interpretation

The paradigm settings mirror those of the original study describing "an attentional blink" with the exception of using 5.6 letters per second compared to the original description of 11.11 letters per second (Raymond et al., 1992). This seminal study demonstrated participants were able to identify both targets if they either occurred in direct succession (T2 within 100 ms of T1) or were separated by a gap of at least 500 ms, of note this study tested university educated adults aged between 22 and 39 years old. There was a significant drop if performance when T2 presented 200 ms to 500 ms after T1, which they termed the "attentional blink". This is the duration required to monitor and recognise a target, disengage and then monitor and detect a new target. The greater the duration of the attentional blink, the weaker cognitive processing power of visual attention. The task requires orienting in time as well as space.

2.4.6 Stroop test

Administration

Participants are presented in the centre of the screen with the name of a colour in a coloured font, and they must identify the colour of the font by pressing the corresponding button on the Cedrus RB-844 response box. There are two conditions. Congruent: In this condition the colour name and the colour of the font are the same. For example when presented with the word "BLUE" printed in blue ink, the correct answer is 'blue' on the response controller. Participants can respond quickly because the word and the font colour match.

Incongruent: In this condition the colour name and the colour of the font differ. For example, the word "BLUE" will be presented in red ink, and the correct answer will depend on inhibiting an automated response (Stroop, 1935).
Following 10 practice trials for each block, for 48 trials participants were required to recognise the meaning of the written word (ignoring the font colour) and for 48 trials to identify to the colour of the font (ignoring the word itself). Half of all trials

were word-font congruent or neutral, the other half of trials were incongruent. A new stimulus was presented 1000 ms following a response. The dependent variables of interest were reaction time.

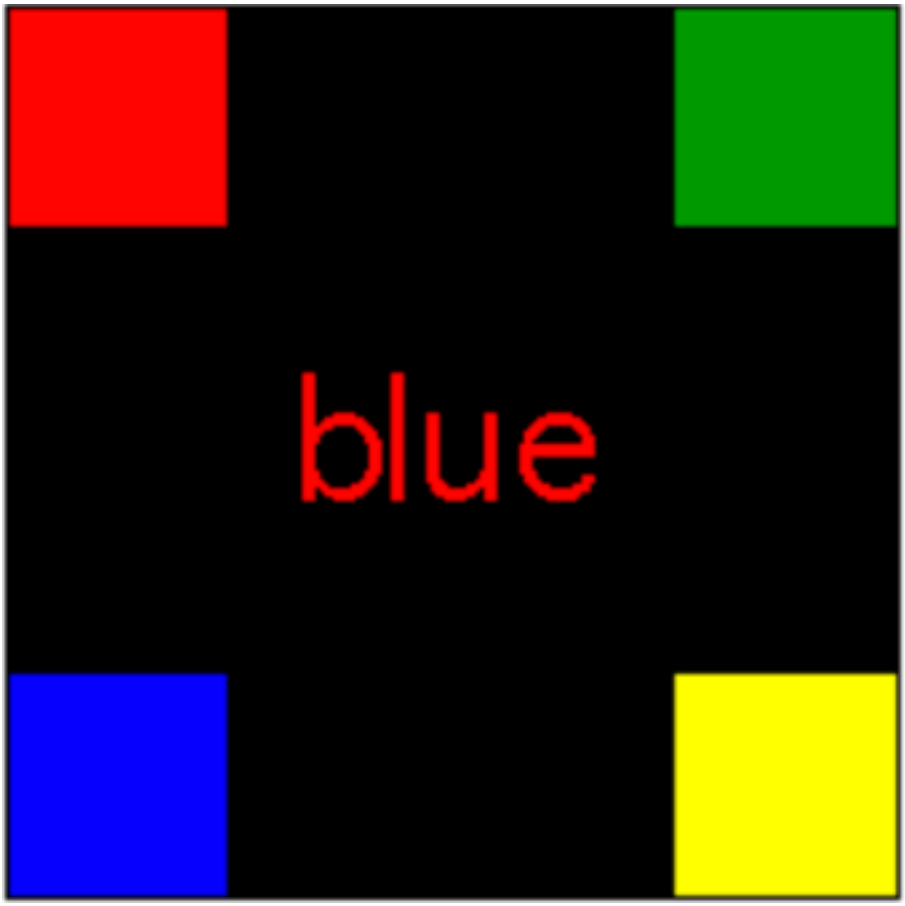

Figure 2.7 An example of the incongruent condition in Stroop task (Durgin, 2000). The target word was presented in a conflicting colour, which the participant matched to a colour on the response box, depicted at each corner of this picture purely for appreciation (Systems, 2018).

Interpretation

The paradigm mirrors the original format formulated by Stroop in 1935 with the exception of participants' responses being recorded via a response box rather than given orally. Reading is an automatized task and as such, puts little stress on cognitive processing, however, when the participants is required to ignore the meaning of the word and identify the ink colour of the font (incongruent condition) reactions times become longer. This is termed the Stroop interference effect and denotes the time required to ignore what is normally processed automatically. A

longer Stroop reaction time represents weaker cognitive processing power for inhibition, an executive function.

2.4.7 Walking while talking

Administration

The walking while talking test is a real world test of attention on daily activities in those with cognitive impairment. The control blocks of the test assess the time taken (in seconds) to walk 20 metres at the subject's fastest walking pace. They would walk 10 metres, turn and return 10 metres from where they came. Subjects are then timed walking the same course as the timed gait, whilst reciting aloud either the letters of the alphabet sequentially or the three times table, for each step taken (WWT). Impaired WWT has been shown to correspond to a risk of falls in the Parkinson's disease population (LaPointe et al., 2010).

Interpretation

Decline in gait is common in older adults and is associated with cognitive and physical decline (Verghese et al., 2002b). Walking while talking differs from walking alone by the requirement to dual task and allocate attention to competing task demands, which is considered an executive function (Holtzer et al., 2005). A decline in the ability to dual task with age has been proposed to be a result of a response selection bottleneck (Hartley, 2001), a decline in processing speed (Salthouse, 1996) and reduced executive attentional resources (Glass et al., 2000).

2.4.8 Practice effect and test-retest reliability (TRR)

This study employed a repeated measures experiment design, which is inherently at risk of practice effects and TRR interfering with a participant's true score. Practice effects occur when repetition leads to improved performance, in the context of standard neuropsychological paradigms this is proposed to relate to explicit recall of test items previously presented (Calamia et al., 2013). This has been demonstrated on verbal memory tasks where minimal improvement was noted on

retesting with alternate versions whereas a significant practice improvement was elicited when the same version was retested (Benedict, 2005).

Aside from using an alternate version of the test another technique to control for practice effects is by allowing participants to practice until their performance has plateaued before applying an intervention. Dual baseline assessments, where 2 practice sessions of a task are completed before testing, have been shown to minimise practice effects (Duff et al., 2001, McCaffrey and Westervelt, 1995), although the degree to which this occurs is task complexity dependent, the simpler the task the sooner performance ceiling is achieved.

TRR is the concept of consistent test performance across different time intervals, this is dependent on measurement error related to variance. TRR is independent of practice effects, for example a test can elicit a large practice effect but still have a high TRR if relative performance within the cohort remains consistent across time (i.e. participants who perform badly within the cohort on the first test, continue to perform badly within the cohort on successive testing) (Lemay et al., 2004). TRR is heterogeneous across different cognitive domain testing with increased reliability on attention compared to memory and executive function tasks (Dikmen et al., 1999). This can be attributed in part to memory and executive function testing requiring a degree of novelty for validity, making them less suitable for repeated neuropsychological testing (Ivnik et al., 1999, Burgess, 1997).

When designing this experiment, where possible practice effects and TRR were taken into consideration. For the rapid serial visual presentation paradigm and walking while talking test, there was a lack of published evidence pertaining to these factors, however, pilot data analysis and subsequent full data analysis has demonstrated nullification of practice effects by dual baseline assessments. The MoCA was not designed to be re-tested following a short interval hence we only perform this test once, as a baseline measure and do not use it to assess response to intervention.

Forward and backward digit span, and simple and choice reaction time are validated (Wilson et al., 2000, Lemay et al., 2004) for longitudinal use due to minimal practice effect, which is nullified by dual baseline assessment and good TRR. Of note simple and choice reaction time error scores and response time variability are not suitable for longitudinal evaluation (Lemay et al., 2004). The Stroop test is used to measure executive attention and has been confirmed to have good TRR for completion time scores (Lemay et al., 2004) with practice effects nullified by dual baseline testing when alternate versions were used (Sacks et al., 1991).

2.4.9 Ecological validity

The computerised testing battery was chosen to individually assess each of the three attentional networks as outlined in the background. Alerting attention was measured by assessing the cognitive reaction time. This is a composite score obtained by subtraction of the simple reaction time from the choice reaction time. If assessed individually motor fluctuation between different testing days, especially in participants with PD, DLB or MS could cause an apparent difference in reaction time score despite there being no difference in attention. However, using a composite score eliminates the motor component of reaction time and allows as assessment of cognitive processing speed. The orienting network was assessed by the rapid serial visual presentation paradigm, which measures so called “attentional blink”. This is a measure of the participant’s ability to attend to and engage in a primary target before disengaging and attending to a second target and requires orienting in time as well as space. Executive attention was assessed by the Stoop task, which measures participants’ reaction times when required to demonstrate cognitive flexibility and inhibition.

Digit span forward was chosen as a measure of working memory and concentration whilst digit span backward was in addition a measure of executive

function. The walking whilst walking test was specifically chosen as a more ecologically valid assessment of attention on an individual's daily function.

The test battery was chosen to align with the Posner-Petersen model of attention, which as already discussed in section 1.2.2, is limited by its neurotransmitter determinism to specific attentional networks. Just as goal derived attentional function is dependent on synergistic neurotransmitter pathways, in contrast to the unitary pathways proposed by Posner-Petersen; likewise neuropsychometric paradigms designed to test a singular attentional network, are rarely pure. I am confident the pairing of the neuropsychometric test with the attentional network is sensitive but acknowledge they lack specificity in representing focal attentional processes (Alvarez and Emory, 2006).

2.5 Experiment design and procedure

We had two iterations of this protocol (version 2, used in chapter 4,5, and 6 was updated after the results obtained in chapter 3)

2.5.1 Protocol Version 1 (Chapter 3)

A double blind, crossover trial compared instant caffeinated coffee with decaffeinated instant coffee (a cup containing 1 standard sachet of 2g Starbucks VIA Italian roast decaffeinated coffee dissolved in 250ml of hot water). The coffee was served with or without artificial sweetener as per patient preference but consistently given across the trial. Milk was not offered. The drink was served at a temperature range of between 50 - 60ºC, which was confirmed by measurement with a thermometer. This ensured the drink was hot but not too hot for safe consumption.

2.5.2 Protocol Version 2 (Chapters 4,5,6)

A single blind, crossover trial compared 100mg caffeine (Proplus) tablets dissolved in instant decaffeinated coffee, with instant decaffeinated coffee. The coffee was served with or without artificial sweetener as per patient preference but consistently given across the trial. Milk was not offered. The drink was served at a temperature range of between 50 - 60ºC, which was confirmed by measurement with a thermometer. This ensured the drink was hot but not too hot for safe consumption.

Analysis of the data from chapter 3 using version 1 of the protocol highlighted a deficiency in the RSVP paradigm settings. The interval between T1 and T2 targets were too long and were not sensitive enough to identify the "attentional blink" accurately. The interval durations were therefore halved as outlined above. The remaining task settings were considered optimal and therefore not adjusted.

2.5.3 Motivation for protocol amendment

In version 1 (chapter 3) the intervention given was a cup containing 1 standard sachet of 2g Starbucks VIA Italian roast caffeinated coffee dissolved in 250ml of hot water. The caffeine content advertised was 130mg per sachet, however, following testing at the University of Bristol chemistry laboratories, the caffeine content was found to be 65mg i.e. half the expected level. Therefore in version 2, for the experiments detailed in chapters 4,5 and 6, the intervention consisted as the same decaffeinated coffee drink as placebo with the addition of a dissolved 100mg Pro Plus caffeine tablet.

The dose has been chosen on the basis it should be high enough to induce a therapeutic effect without risk of significant side effects. Using Pro Plus caffeine added to decaffeinated coffee allowed a reproducible dose within the caffeinated group and a reproducible flavour between the caffeinated and decaffeinated group.

However, given the caffeine tablets were required to be crushed and then dissolved into the decaffeinated coffee, the trial changed from double blind to single blind.

2.5.4 Randomisation and blinding

The randomisation occurs on day 8 when the participant will receive either caffeinated or decaffeinated coffee. On day 9 they received the alternative coffee type to day 8. Participants entering the trial were alternately allocated to caffeinated or decaffeinated coffee on day 8. This ensured 50% of participants received decaffeinated coffee on day 8 followed by caffeinated coffee on day 9 whilst the other 50% were counter balanced and received caffeinated coffee on day 8 and decaffeinated coffee on day 9. Equal counter balance was important to prevent imbalance of covariates and allow statistical analysis to assess whether drink order affected performance.

2.6 Data analysis

All raw data was initially pre processed in Microsoft Excel 2011 for Mac before being transferred to IBM SPSS version 23 for Mac for statistical processing.

The 4 computerised paradigms all elicited reaction time data. Any responses with a reaction time less than 200ms was excluded as these were assumed to be anticipatory rather than reactionary, however, no such data points were recorded. Reaction times greater than 5 seconds would automatically time out as part of the computer programme setting to factor in participants pausing to consume a drink of water or make themselves more comfortable.

Outliers were not excluded from data analysis due to the repeated measures design negating a skewing effect on the data, provided the outlier was equally deviated

from the group mean on both intervention and control results. Therefore no sample data was excluded.

2.6.1 Comparing the effect of caffeine versus placebo (chapters 3,4,5,6)

For digit span, simple reaction time, choice reaction time, Stroop task and walking while talking task, the independent and dependent variables are the same and therefore the data can be analysed following the same algorithm as outlined below.

The cross over design of the trial makes a paired samples t-test the most appropriate statistical analysis provided certain assumptions are met. Inherent study design requirements include (i) a continuous variable, which in this case is time and (ii) a singular independent variable being categorical with two related groups, which is intervention (caffeine or placebo given) to the same person at different time points. The most important assumption is of approximate normality on the *differences* between the paired values (rather than the absolute values of the paired groups). These are obtained by subtracting the placebo score from the caffeine score for each participant and then assessing these results for normality. The paired samples t-test is considered fairly robust to violations of normality (Parametric).

Normality is assessed visually using a Normal Q-Q plot and numerically using the Shapiro-Wilk test of normality, which is recommended for sample sizes less than 50, as it will report minor deviations from normality as significant in large sample sizes. The null hypothesis for the Shapiro-Wilk test is that the data is normally distributed. Therefore if the significance value is greater than 0.05, the null hypothesis is accepted and the data can be considered normally distributed. Conversely if the significance value is less than or equal to 0.05 then the null hypothesis is rejected and the data is considered non parametric.

If the data is normally distributed a paired samples t-test will be used. If the data is non parametric then the Wilcoxon signed-rank test will be used if the data is symmetrically distributed or the Sign test will be used if the data is asymmetrically distributed (Statistics, 2015).

The Rapid Serial Visual Presentation task has three within subjects factors consisting of (i) task (single or dual), (ii) interval between target 1 and target 2, and (iii) condition (intervention or placebo). It is therefore unsuitable for a paired samples t-test as there is more than 1 independent variable and would be most suitable for statistical analysis by a repeated measures ANOVA (analysis of variance) test provided certain assumptions are met. Inherent study design requirements include (i) a continuous variable, which is percentage of correct target identification and (ii) the independent variable consisting of at least two or more categorical levels. As with the paired samples t-test the data should be approximately normally distributed but is robust to violations of normality too. The most important assumption is of equal variance between the groups assessed using Mauchly's test of sphericity (Keselman et al., 1980).

The null hypothesis for Mauchly's test of sphericity is the variance of the differences between levels of within subject factors are equal. Therefore if the significance value is greater than 0.05, the null hypothesis is accepted and the data can be considered to have variances of the differences which are equal. Conversely if the significance value is less than or equal to 0.05 then the null hypothesis is rejected and the data is considered to have variances of the differences that are unequal.

If the data demonstrate equal variances of the differences then the standard ANOVA output will be appropriate, however, if sphericity is not assumed then the Greenhouse-Geisser output should be interpreted.

2.6.2 Comparing dementia with Lewy bodies (chapters 3) or Parkinson's disease with aged matched controls (chapters 5)

The analysis for this data set is very similar to that described in 2.6.1 *Comparing the effect of caffeine versus placebo.* The fundamental difference is a change from a repeated measures sample to two independent samples. For digit span, simple reaction time, choice reaction time, Stroop task and walking while talking task, if normally distributed they are analysed by an independent samples t-test and if non parametric they are analysed by a Welch's t-test. The Rapid Serial Visual Presentation task is analysed by a three way ANOVA.

2.6.3 Co-variables

The effect of age, sex, MoCA score, sleep and baseline caffeine consumption were assessed using correlation. The best way of assessing for a correlation between two variables is to draw a scatterplot and visually assess for a monotonic relationship. The data should be assessed for normality using Shapiro-Wilk's test outlined above. For co-variables that are continuous, age, MoCA score, sleep and habitual caffeine intake, provided the scatterplots demonstrate a monotonic relationship, if the data are normally distributed then a Pearson product-moment correlation should be used, however, if the data is non parametric then a Spearman's rank-order correlation should be used (Statistics, 2016).

Both the Pearson product-moment correlation and the Spearman's rank-order correlation are used to assess the strength of a linear relationship between two continuous variables. The test generates a correlation coefficient with values ranging from +1, a perfect positive linear relationship to -1, a perfect negative linear relationship. The closer the value lies to zero, the weaker the relationship between the two variables. The null hypothesis is the correlation coefficient is equal to zero. Therefore if the significance value is greater than 0.05, the null hypothesis is accepted and the two variables can be considered as not correlating. Conversely if

the significance value is less than or equal to 0.05 then the null hypothesis is rejected and the two variables are considered to correlate with each other.

Sex, as a dichotomous variable does not conform to the assumptions of the tests described above. If the data is normally distributed and there is homogeneity of the variances then a point-biserial correlation calculation is appropriate. However, if the data is non parametric or there is heterogeneity of the variances then the Kendall's tau-b correlation would be appropriate.

Both the point-biserial correlation and the Kendall's tau-b correlation generate a correlation coefficient with values ranging from +1, a perfect positive linear relationship to -1, a perfect negative linear relationship. The closer the value lies to zero, the weaker the relationship between the two variables. The null hypothesis is the correlation coefficient is equal to zero. Therefore if the significance value is greater than 0.05, the null hypothesis is accepted and the two variables can be considered as not correlating. Conversely if the significance value is less than or equal to 0.05 then the null hypothesis is rejected and the two variables are considered to correlate with each other.

2.6.4 Effect size

Calculation of an effect size allows an estimation of the size of intervention effect relative to the difference expected by chance. Effect size was calculated using Cohen's d, the mean difference between the two repeated measures is divided by the standard deviation of the difference. The effect size can be characterised as small d=0.2, medium d=0.5 and large d=0.8 (Cohen, 1992).

2.6.5 Power

Study power is the probability one will reject a false null hypothesis and find an effect when one is present. It equates to 1 – type II error. It is dependent on the alpha level, sample size and effect size. G*Power is a validated statistical package

(Faul et al., 2007) designed to compute power analyses and will be used for each of the statistical analyses described above.

Chapter 3

The utility of caffeine as an attentional enhancer in dementia with Lewy bodies and healthy elderly participants

This chapter contains extracts from published work:
Sharma, K., Davis, T., & Coulthard, E. (2016). Enhancing attention in neurodegenerative diseases: current therapies and future directions. Translational Neuroscience, 7(1), 98-109.
I have only included parts of the manuscript I personally wrote, I have not included text written by T Davis. E Coulthard contributed in a supervisory role only.

3.1 Introduction

In 2015 David Cameron set out the "Prime Minister's challenge on dementia 2020" (Health, 2015). This Department of Health white paper outlined the challenge not just for the UK but globally, associated with an exponential rise in dementia care costs associated with an aging population. A need to develop more research in this field is imperative, especially in the domain of symptomatic treatments where a paucity of options prevails.

Dementia with Lewy bodies (DLB) is characterised by fluctuations in consciousness leading to daytime somnolence, visual hallucinations and Parkinsonism with additional features such as REM sleep behaviour disorder. Parkinson's disease progresses to dementia in up to 80% (Emre et al., 2007b). These two clinical syndromes differ in the sequence of onset of dementia and Parkinsonism, but with progression both syndromes and underlying pathological

changes completely overlap and can be viewed as a continuum rather than dichotomous entities. They are known under the umbrella term Lewy body dementias (Walker et al., 2015).

People with DLB struggle to attain the minimal activation of alertness needed for both attention and information processing to operate (Ballard et al., 2001). DLB patients also experience serious difficulties in drawing their attention to new relevant locations, suggesting their orienting attention is impaired (Cormack et al., 2004). Executive dysfunction is an early, prominent neuropsychological feature (Collerton et al., 2003), thus failure of attention is a particular problem in this group with all networks affected (Calderon et al., 2001).

Anecdotally as clinicians, we have seen people so profoundly affected by attention fluctuations that they are admitted to hospital with episodes of presumed loss of consciousness and investigated for epilepsy and other conditions. A breakdown in attentional function is thought to underpin the tendency to fluctuations which may also contribute to the development of visual hallucinations through impaired bottom-up processing of sensory information which allows false data to be sent to the entire cortex and not be recognised as abnormal (Heitz et al., 2015).

DLB results from the accumulation of neuronal intracellular aggregates of a-synuclein, which form Lewy bodies, secondary cellular injury and apoptotic neurodegeneration (McKeith et al., 2005). Pathologically, the concentration of Lewy bodies is distributed in the frontal, cingulate and inferior temporal cortex, substantia nigra, locus coeruleus and components of the basal forebrain cholinergic system (McKeith et al., 1996). The distribution of cerebral pathology can be affiliated to the trinity of attentional networks, alerting, orienting and executive. The observed deficits in alerting attention correspond to pathology in the locus coeruleus affecting the noradrenergic system; orienting attention deficits corresponds to the cholinergic system of the basal forebrain and executive attention

deficits correspond to substantia nigra pathology affecting the dopaminergic system (Fuentes et al., 2010).

A modified Attention Network Test has been used to assess the efficiency of the three attentional networks in DLB with aged matched controls. This confirmed impaired alerting and executive attention with relatively preserved orienting attention. Interestingly there was no correlation between grey or white matter brain volume with either alerting or executive performance, raising the possibility of network dysfunction rather than a focal structural abnormality as the underlying substrate (Cromarty et al., 2018). This concept has been strengthened by functional magnetic resonance imaging demonstrating hypoconnectivity between different attention networks in DLB whilst performing the Attention Network Test (Kobeleva et al., 2017).

Using medications to enhance attention in this population can consequentially improve other cognitive domains such as memory as well as overall cognitive function (Chun and Turk-Browne, 2007, Souza et al., 2014). The net effect to an individual is an improved quality of life and maintenance of independence a few years longer than previously possible (Geldmacher et al., 2003). Across a population of people with dementia this will significantly reduce care costs, potentially saving millions of pounds each year. The extensive cholinergic depletion in DLB may explain (Collerton et al., 2003) improvement with cholinesterase inhibitor therapy (Wesnes et al., 2005) which has been licenced (specifically Rivastigmine) for Parkinson's disease dementia since 2006 (Emre et al., 2004) and is used in DLB on the basis of the same underlying pathology.

There is no established effective therapy to improve daytime attention and somnolence, which has a significant impact on quality of life. Stimulants such methylphenidate, dextroamphetamine and modafinil have been explored with mixed success (Seppi et al., 2011, Hogl et al., 2002). Worryingly over stimulation has the possibility to precipitate psychosis (Prado et al., 2012). This is perhaps no

surprise considering the Yerkes-Dodson law suggests that optimal stimulation is not the same as maximal stimulation (Yerkes and Dodson, 1908). The ideal level of stimulation is yet to be elucidated but it is clear that strong stimulants, such as those described above, have not produced the cognitive benefits they initially promised. It may seem counter intuitive to conventional reasoning but a weak rather than a strong stimulant may provide greater benefits.

3.1.1 Aims

1. To assess whether caffeine compared to placebo improves attention in fully withdrawn healthy elderly participants on computerised neuropsychology paradigms and functional tasks of attention.
2. To assess whether caffeine compared to placebo improves attention in fully withdrawn DLB participants on computerised neuropsychology paradigms and functional tasks of attention.

3.1.2 Hypothesis

1. Acute caffeine ingestion will improve attention in the alerting and executive domain in healthy elderly people.
2. Acute caffeine ingestion will improve attention in the alerting and executive domain in people with DLB.
3. Healthy aged matched participants will perform better than DLB participants on tests of alerting, orienting and executive attention, walking and walking while talking tasks.

3.2 Methods

3.2.1 Participants

Twenty healthy elderly and six DLB participants were tested. They were recruited from a clinical research database held in North Bristol NHS Trust.

The inclusion criteria for healthy elderly participants were:

- adequate vision to perform the tasks
- an adequate level of communication in written and verbal English
- independently mobile

The exclusion criteria for healthy participants were:

- any concomitant serious illness likely to interfere with cognitive or physical performance
- any reported cognitive problems
- signs of cognitive impairment (e.g. Montreal Cognitive Assessment <23)
- inability to consent to research, in keeping with the Mental Capacity Act 2005

The inclusion criteria for DLB participants were:

- an established diagnosis of DLB
- adequate vision to perform the tasks
- an adequate level of communication in written and verbal English
- independently mobile

The exclusion criteria for DLB participants were:

- any concomitant serious illness likely to interfere with cognitive or physical performance

- inability to consent to research, in keeping with the Mental Capacity Act 2005
- loss of capacity to consent to research during the trial

	DLB	Controls
Participants	6	20
Age	73.0 (67-81)	71.1 (52-87)
Sex	5 male : 1 female	6 male : 14 female
Baseline MoCA	19.17 (14-23)	27.75 (24-30)
Habitual daily caffeine intake (mg)	44 (0-85)	86 (0-170)
Taking acetylcholinesterase inhibitors	6 (100%)	0
Taking dopaminergic medication	4 (67%)	0

Table 3.1 Comparison of DLB and healthy control participant demographics

All participants had been stable on their current medication for at least three months and there were no medication changes during the trial.

DLB Recruitment

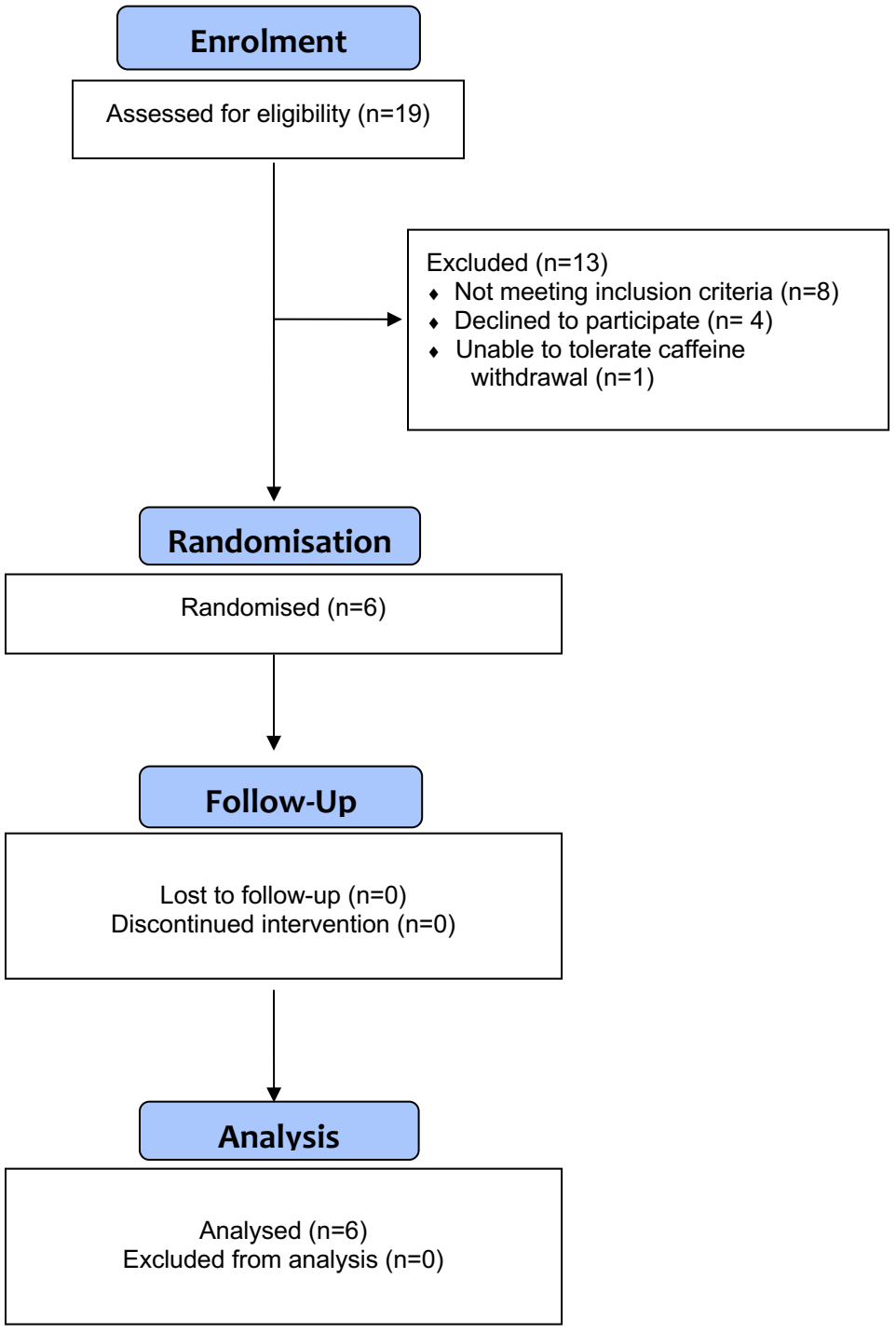

Figure 3.1a Recruitment phases for DLB participants in this placebo controlled, cross over trial. Adapted from Consolidated Standards of Reporting Trials Group (Moher et al., 2001)

Healthy Participant Recruitment

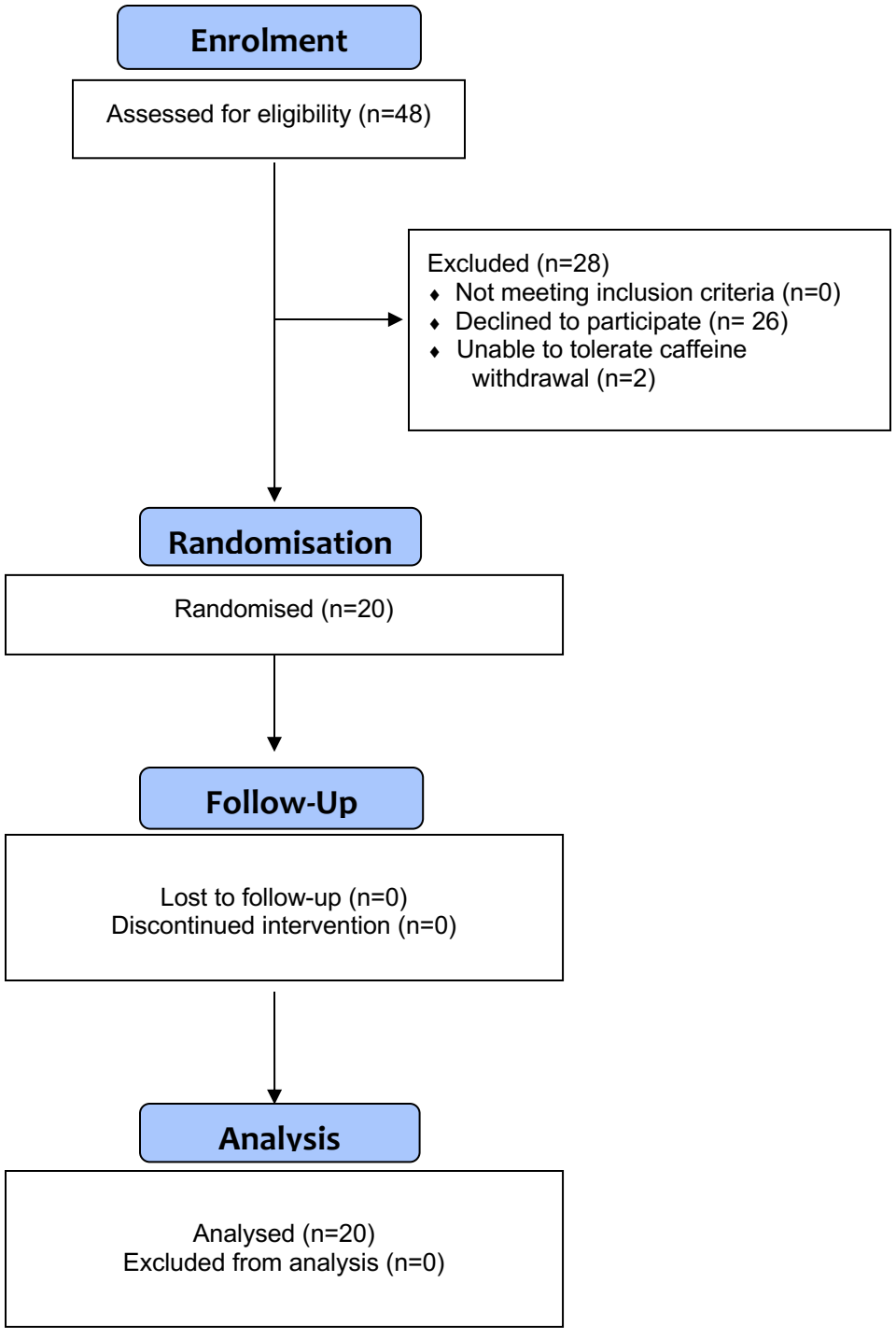

Figure 3.1b Recruitment phases for healthy elderly in this placebo controlled, cross over trial participants. Adapted from Consolidated Standards of Reporting Trials Group (Moher et al., 2001)

3.2.2 Procedure

A double blind, crossover trial compared instant coffee (a cup containing 1 standard sachet of approximately 2g Starbucks VIA Ready Brew Italian Roast with 250ml of hot water) with decaffeinated instant coffee (a cup containing 1 standard sachet of approximately 2g Starbucks VIA Ready Brew Decaffeinated Italian Roast with 250ml of hot water) with or without artificial sweetener as per patient preference but consistently given across the trial. The dose was chosen on the basis it would contain 135mg of caffeine (the actual dose was 62mg see section 2.5.3 for further details), which from trial data should be high enough to induce a therapeutic effect without risk of significant side effects. The sachets come in a standard weight and are the same flavour therefore using sachets should allow a reproducible dose within the caffeinated group and reproducible flavour between the caffeinated and decaffeinated group. The coffee was served at a temperature range of between 50 - 60ºC which was confirmed by measurement with a thermometer, to ensure the drink was hot but not too hot for safe consumption.

A member of the dementia research group not involved in the trial, separated sachets of caffeinated and decaffeinated coffee required for the experiment and covered them individually in masking tape making them unidentifiable. All coffee sachets of one type were labelled with a sticky label inscribed with the letter A and all coffee sachets of the other type with a sticky label inscribed B. No members of the research team were informed which letter corresponded to which coffee type but a written record was kept in a sealed envelope.

Stratified equal group random allocation for each block of patient groups i.e. healthy participants and those with DLB. Using http://www.randomizer.org to generate 40 random numbers between 1 and 99, we took take odd and even numbers to indicate intervention A and B respectively. Once an intervention letter accrued half of the block sample population then the remaining participants in that block received the other intervention first.

Participants attended for baseline testing on day 1 without any dietary caffeine restriction. Following testing they were given a supply of either decaffeinated coffee and/or decaffeinated tea to cover the trial duration (as per their consumption preference) and requested to not ingest caffeine containing foods such as tea, coffee, chocolate etc. for the remainder of the trial (9 days) but could freely consume the decaffeinated tea/coffee we supplied them. On day seven (i.e. 1 week free from caffeine) participants repeated testing to assess for effects of caffeine withdrawal on attention and allow task familiarisation so that the effect of learning on subsequent performance was minimised. On day eight participants received either caffeinated or decaffeinated coffee and testing started 60 minutes later. In the interim, participants would wait in a quiet waiting room with books and magazines for interest if desired. On day nine the participants received the alternative type of coffee (caffeinated or decaffeinated whichever not already had) and began testing 60 minutes following consumption. Testing was performed within 15 minutes of the same time on all days.

3.2.3 Task

The task battery consisted of:

i. The Montreal Cognitive Assessment (MoCA)
ii. Digit span
iii. Simple reaction time
iv. Choice reaction time
v. The rapid serial visual presentation (RSVP) paradigm
vi. Stroop task
vii. Walking while talking test (WWT)

3.3 Results

3.3.1 Alerting attention

Healthy Elderly

A paired-samples t-test was used to determine whether there was a statistically significant mean difference between caffeine versus placebo on ***simple reaction time*** scores. Data are mean ± standard deviation, unless otherwise stated. The assumption of normality was not violated, as assessed by Shapiro-Wilk's test (p = 0.18). There was no statistically significant change between reaction time whilst on caffeine (303 ms ± 45) compared to placebo (307 ms ± 456), -3 ms, 95% CI [-18, 12](Cunha, 2005), t(19) = -0.41, p = 0.69, d = -0.09.

A paired-samples t-test was used to determine whether there was a statistically significant mean difference between caffeine versus placebo on ***choice reaction time*** scores. Data are mean ± standard deviation, unless otherwise stated. The assumption of normality was not violated, as assessed by Shapiro-Wilk's test (p = 0.35). There was no statistically significant change between reaction time whilst on caffeine (535 ms ± 71) compared to placebo (525 ms ± 59), 10 ms, 95% CI [-7, 28], t(11) = 1.24, p = 0.23, d = 028.
There was no correlation to age, sex, MoCA score or habitual caffeine intake.

A sign test with continuity correction was used determine whether there was a statistically significant median difference between caffeine versus placebo on ***choice reaction time*** error rates. Of the 20 participants recruited to the study, caffeine ingestion compared to placebo reduced errors in 4 participants, increased errors in 9 participants and had no effect on 7 participants. There was no statistically significant difference between errors whilst on caffeine (Median = 0.01) compared to placebo (Median = 0.01), 0.00, z = 1.11, p = 0.27.
There was no correlation to age, sex, MoCA score or habitual caffeine intake.

A Wilcoxon signed-rank test was used determine whether there was a statistically significant median difference between caffeine versus placebo on ***cognitive reaction time***. The difference scores were symmetrically distributed, as assessed by a histogram. Of the 20 participants recruited to the study, caffeine improved cognitive reaction time in 6 participants and worsened cognitive reaction time in 14 participants. There was no statistically significant difference between cognitive reaction time whilst on caffeine (Median = 240) compared to placebo (Median = 224), 16 ms, z = -1.68, p = 0.09.

There was no correlation to age, sex, MoCA score or habitual caffeine intake.

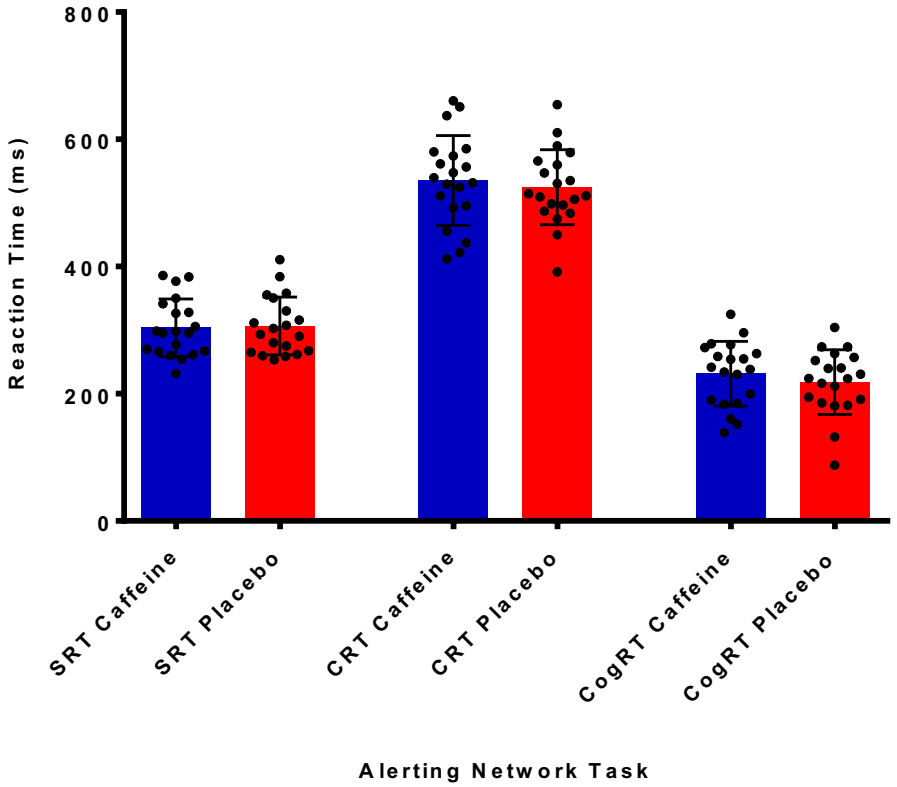

Figure 3.2a Healthy elderly mean reaction time on simple reaction time (SRT), choice reaction time (CRT) and cognitive reaction time (CogRT). No significant difference was observed.

<u>Dementia with Lewy bodies</u>

A paired-samples t-test was used to determine whether there was a statistically significant mean difference between caffeine versus placebo on ***simple***

reaction time scores. Data are mean ± standard deviation, unless otherwise stated. The assumption of normality was not violated, as assessed by Shapiro-Wilk's test (p = 0.28). There was no statistically significant change between reaction time whilst on caffeine (457 ms ± 113) compared to placebo (435 ms ± 95.), 23 ms, 95% CI [-73, 118], t(5) = 0.61, p = 0.57, d = 0.25.

A paired-samples t-test was used to determine whether there was a statistically significant mean difference between caffeine versus placebo on ***choice reaction time*** scores. Data are mean ± standard deviation, unless otherwise stated. The assumption of normality was not violated, as assessed by Shapiro-Wilk's test (p = 0.94). There was no statistically significant change between reaction time whilst on caffeine (883 ms ± 189) compared to placebo (868 ms ± 186), 14 ms, 95% CI [-110, 138], t(5) = 0.30, p = 0.78, d = 0.12.

A paired-samples t-test was used to determine whether there was a statistically significant median difference between caffeine versus placebo on ***choice reaction time*** error rates. The assumption of normality was not violated, as assessed by Shapiro-Wilk's test (p = 0.51). There was no statistically significant change between error rate whilst on caffeine (3% ± 2.32) compared to placebo (3% ± 2.88), 0.33%, 95% CI [-1, 2], t(5) = 0.50, p = 0.64, d = 0.20.

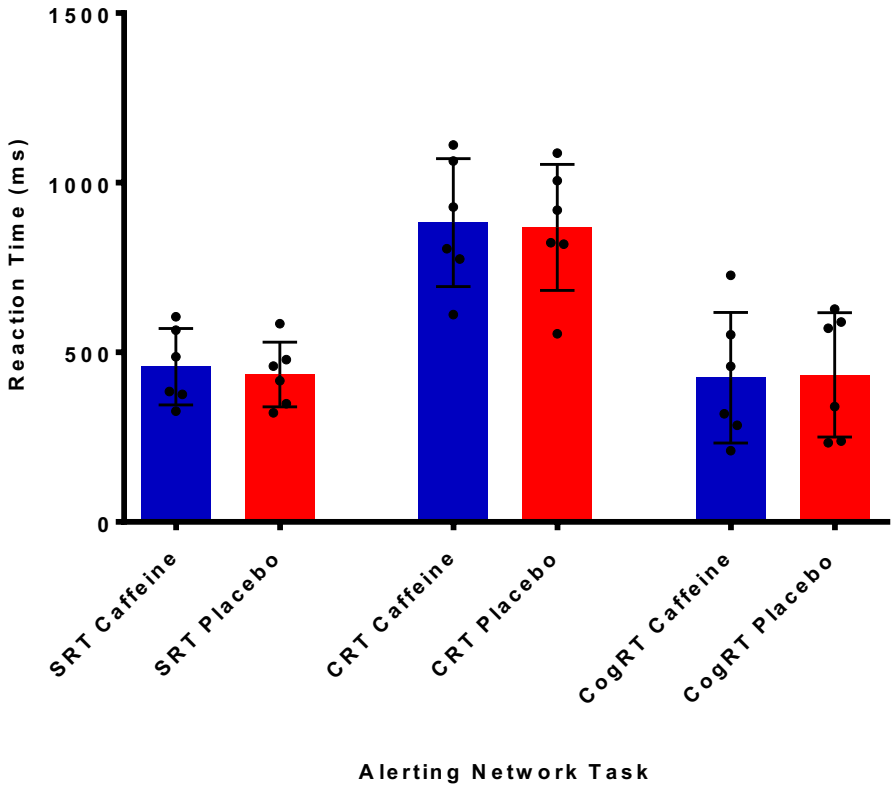

Figure 3.2b DLB mean reaction time on simple reaction time (SRT), choice reaction time (CRT) and cognitive reaction time (CogRT). No significant difference was observed.

A paired-samples t-test was used to determine whether there was a statistically significant mean difference between caffeine versus placebo on ***cognitive reaction time*** scores. Data are mean ± standard deviation, unless otherwise stated. The assumption of normality was not violated, as assessed by Shapiro-Wilk's test (p = 0.83). There was no statistically significant change between reaction time whilst on caffeine (425 ms ± 193) compared to placebo (433 ms ± 183), -8 ms, 95% CI [-114, 97], t(5) = -0.20, p = 0.85, d = -0.08.

3.3.2 Orienting attention

Healthy Elderly

A three-way repeated measures ANOVA was run to determine the effect of caffeine on accuracy at different time points on the Rapid Serial Visual presentation task. Epsilon (ε) was 0.49, as calculated according to Greenhouse & Geisser (1959),

and was used to correct the one-way repeated measures ANOVA. There was no statistically significant three-way interaction between the three main effects of caffeine, task and time, $F(1.95, 31.24) = 0.91$, $p = 0.41$.

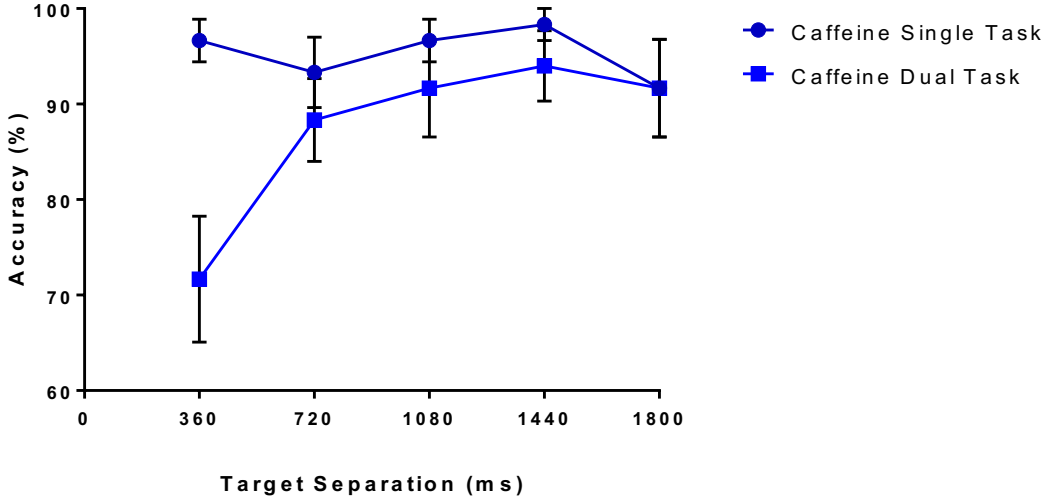

Figure 3.3a Healthy elderly mean accuracy on single and dual tasks of the Rapid Serial Visual Presentation paradigm whilst on caffeine. Error bars represent standard error of the mean. The area of interest is the point of intersect between single and dual task result lines. This represents the "attentional blink", the time required to attend a primary target before disengaging and attending to a second target accurately. Under caffeine the attentional blink is 1860 ms.

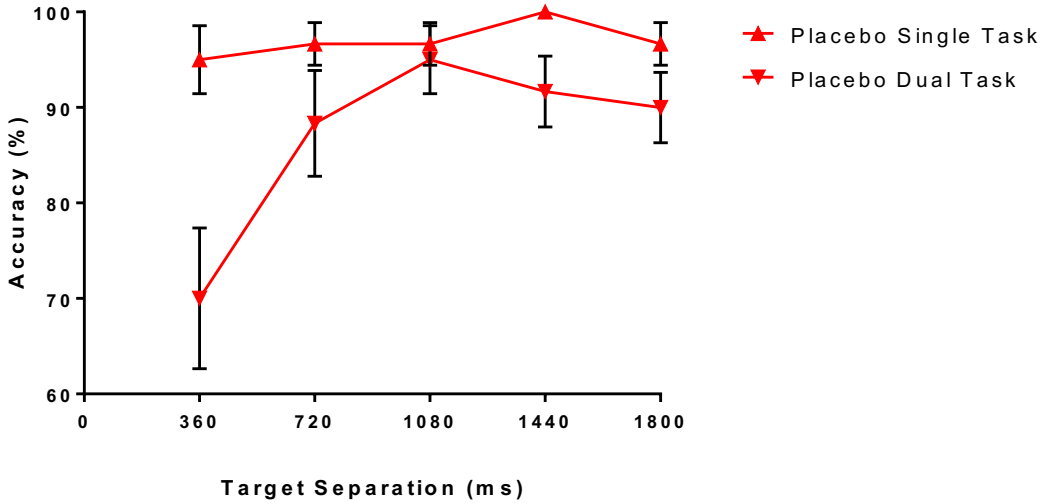

Figure 3.3b Healthy elderly mean accuracy on single and dual tasks of the Rapid Serial Visual Presentation paradigm whilst on placebo. Error bars represent standard error of the mean. Under placebo the attentional blink is 1080 ms.

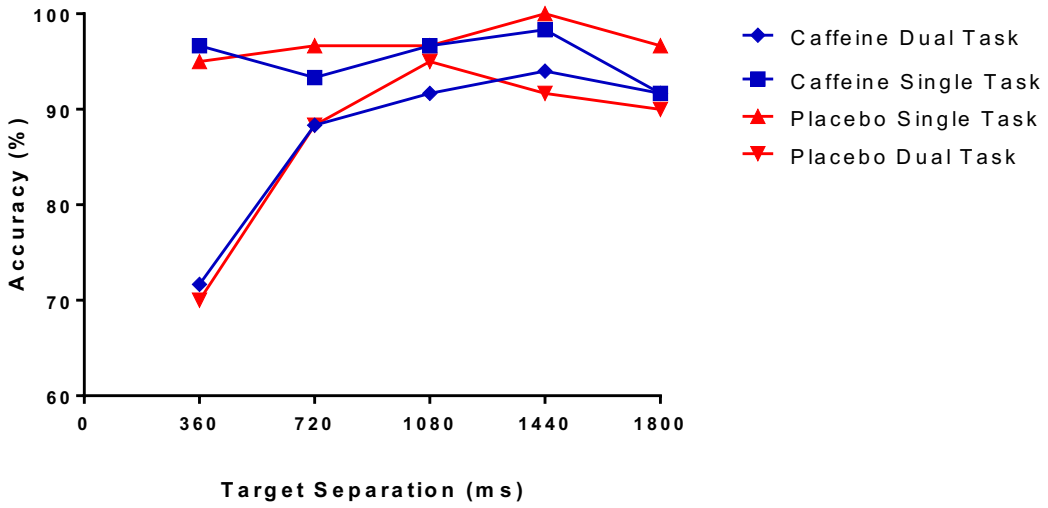

Figure 3.3c Healthy elderly mean accuracy on single and dual tasks of the Rapid Serial Visual Presentation paradigm comparing caffeine with placebo.

Dementia with Lewy bodies

A three-way repeated measures ANOVA was run to determine the effect of caffeine on accuracy at different time points on the Rapid Serial Visual presentation task. Mauchly's test of sphericity indicated that the assumption of sphericity was met for the two-way interaction, $\chi 2(6) = 10.71$, $p = 0.35$. There was no statistically significant three-way interaction between caffeine, task and time, $F(4, 20) = 2.39$, $p = 0.09$.

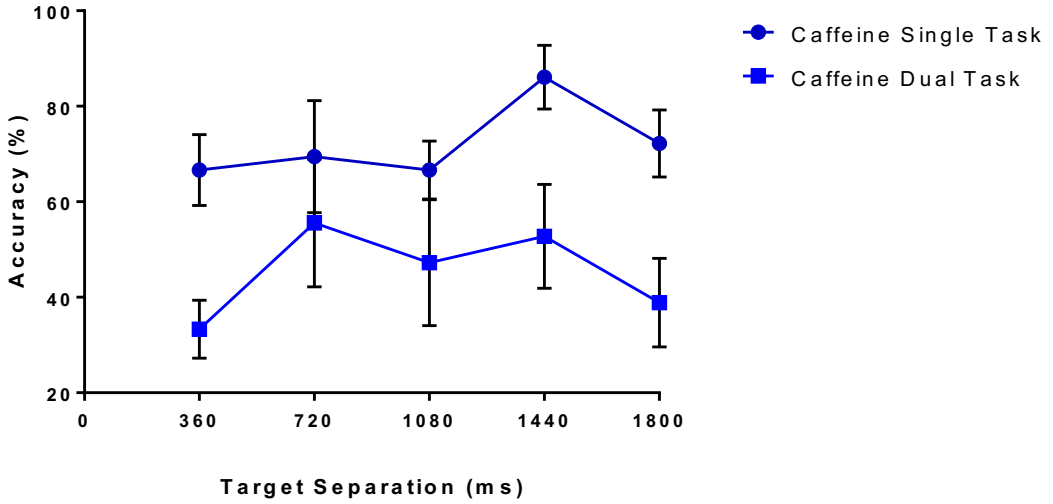

Figure 3.3d DLB mean accuracy on single and dual tasks of the Rapid Serial Visual Presentation paradigm whilst on caffeine. The attentional blink is not observed.

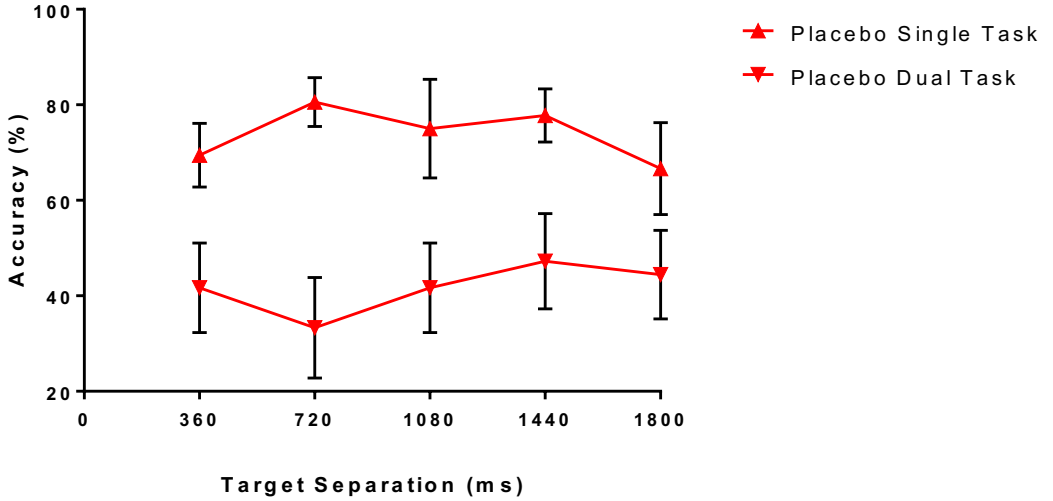

Figure 3.3e DLB mean accuracy on single and dual tasks of the Rapid Serial Visual Presentation paradigm whilst on placebo. The attentional blink is not observed.

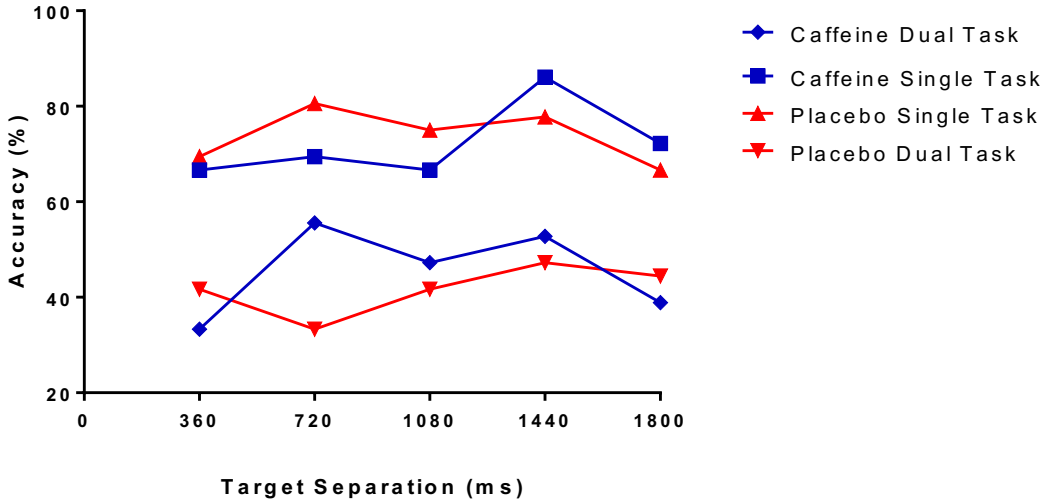

Figure 3.3f DLB mean accuracy on single and dual tasks of the Rapid Serial Visual Presentation paradigm comparing caffeine with placebo.

3.3.3 Executive attention

Healthy Elderly

A paired-samples t-test was used to determine whether there was a statistically significant mean difference between caffeine versus placebo on ***Stroop reaction time*** scores. Data are mean ± standard deviation, unless otherwise stated. The assumption of normality was not violated, as assessed by Shapiro-Wilk's test for incongruent ($p = 0.68$) condition, however, the neutral ($p = 0.00$) and total ($p = 0.03$) conditions were not normally distributed.

There was no statistically significant change in reaction time during the incongruent condition whilst on caffeine (1070 ms ± 280) compared to placebo (1057 ms ± 275), 12 ms, 95% CI [-49, 74], $t(19) = 0.43$, $p = 0.68$, $d = 0.10$.

A sign test with continuity correction was used determine whether there was a statistically significant median difference between caffeine versus placebo on neutral condition reaction time on the Stroop task. Of the 20 participants recruited to the study, caffeine ingestion compared to placebo reduced (quickened) reaction

time in 12 participants and increased reaction time in 8 participants. There was no statistically significant difference between reaction times whilst on caffeine (Median = 863) compared to placebo (Median = 881), -18, z = -0.67, p = 0.50.

A sign test with continuity correction was used determine whether there was a statistically significant median difference between caffeine versus placebo on total Stroop reaction time on the Stroop task. Of the 20 participants recruited to the study, caffeine ingestion compared to placebo reduced (quickened) reaction time in 10 participants and increased reaction time in 10 participants. There was no statistically significant difference between reaction times whilst on caffeine (Median = 1886) compared to placebo (Median = 1955), -68, z = 0.00, p = 1.00.

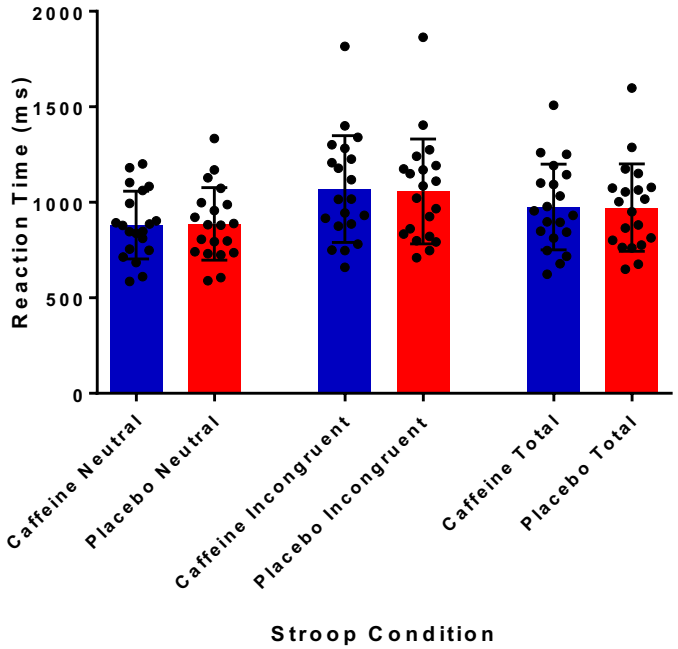

Figure 3.4a Healthy elderly reaction time performance on the Stroop task. No significant difference was observed.

Dementia with Lewy bodies

A paired-samples t-test was used to determine whether there was a statistically significant mean difference between caffeine versus placebo on ***Stroop reaction time*** scores. Data are mean ± standard deviation, unless otherwise stated. The assumption of normality was not violated, as assessed by Shapiro-Wilk's test for neutral (p = 0.76), incongruent (p = 0.78) and total (p = 0.99) conditions.

There was no statistically significant change in reaction time during the neutral condition whilst on caffeine (2091 ms ± 604) compared to placebo (1963 ms ± 499), 128 ms, 95% CI [-311, 566], t(5) = 0.75, p = 0.49 d = 0.31.

There was no statistically significant change in reaction time during the incongruent condition whilst on caffeine (2891 ms ± 1263) compared to placebo (2722 ms ± 375), 169 ms, 95% CI [-384, 723], t(5) = 0.79, p = 0.47, d = 0.32.

There was no statistically significant change in average Stroop reaction time whilst on caffeine (2491 ms ± 920) compared to placebo (2343 ms ± 703), 149 ms, 95% CI [-321, 618], t(5) = 0.81, p = 0.45 d = 0.33.

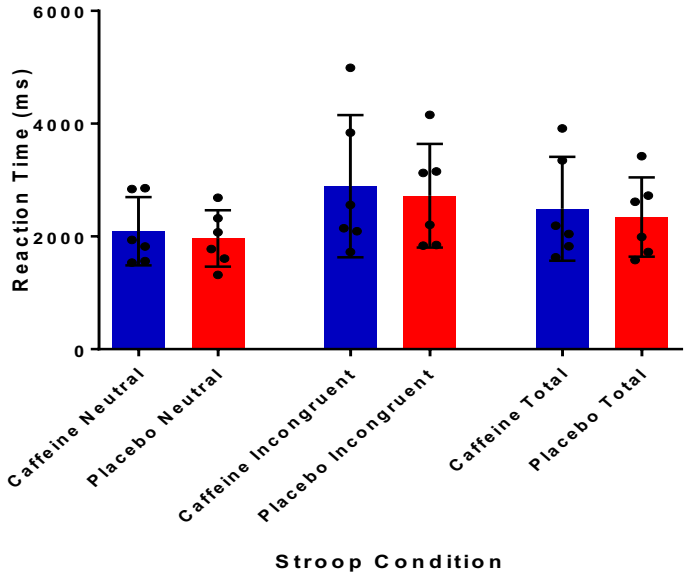

Figure 3.4b DLB reaction time performance on the Stroop task. No significant difference was observed.

3.3.4 Digit Span

Healthy Elderly

A paired-samples t-test was used to determine whether there was a statistically significant mean difference between caffeine versus placebo on ***digit span score***s. Data are mean ± standard deviation, unless otherwise stated.
The assumption of normality was not violated, as assessed by Shapiro-Wilk's test for forward ($p = 0.18$) or total ($p = 0.09$) conditions, however, the backwards ($p = 0.03$) condition was not normally distributed.

There was no statistically significant change in digit span during forward digit span whilst on caffeine (10.6 digits ± 2.3) compared to placebo (11.2 digits ± 2.0), -0.6 digits, 95% CI [-1.3, 0.7], $t(19) = -1.64$, $p = 0.12$, $d = -0.37$.

There was no statistically significant change in total digit span whilst on caffeine (19.7 digits ± 4.5) compared to placebo (21.0 digits ± 4.8), -1.3 digits, 95% CI [-2.6, 0.0], $t(19) = -2.16$, $p = 0.04$, $d = -0.48$.

A sign test with continuity correction was used determine whether there was a statistically significant median difference between caffeine versus placebo on backward digit span. Of the 20 participants recruited to the study, caffeine ingestion compared to placebo reduced (worsened) backward digit span in 7 participants, increased reaction time in 9 participants and had no effect on 4 participants. There was no statistically significant difference between reaction times whilst on caffeine (Median = 8.5) compared to placebo (Median = 10.0), -1.5, $z = 0.25$, $p = 0.80$.

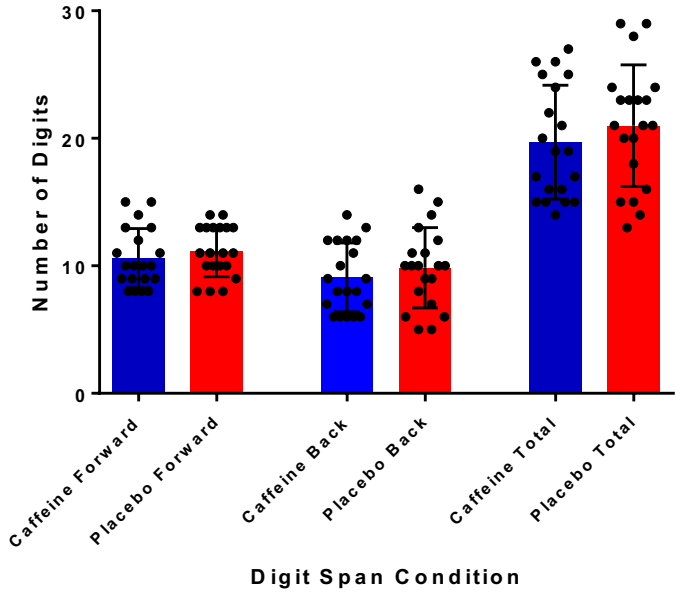

Figure 3.5a Healthy elderly digit span performance. Error bars represent standard error of the mean. No significant difference was observed.

Dementia with Lewy bodies

A paired-samples t-test was used to determine whether there was a statistically significant mean difference between caffeine versus placebo on ***digit span score***s. Data are mean ± standard deviation, unless otherwise stated.
The assumption of normality was not violated, as assessed by Shapiro-Wilk's test for forward ($p = 0.24$), backward ($p = 0.22$) and total ($p = 0.09$) conditions.

There was no statistically significant change in forward digit span whilst on caffeine (8.5 digits ± 1.6) compared to placebo (9.5 digits ± 2.1), -1.0 digits, 95% CI [-2.8, 0.8], $t(5) = -1.46$, $p = 0.20$, $d = -0.60$.

There was no statistically significant change in backward digit span whilst on caffeine (5.2 digits ± 1.8) compared to placebo (5.3 digits ± 2.7), -0.2 digits, 95% CI [-2.0, 1.6], $t(5) = -0.24$, $p = 0.82$, $d = -0.10$.

There was no statistically significant change in total digit span whilst on caffeine (13.7 digits ± 3.1) compared to placebo (14.8 digits ± 3.6), -1.2 digits, 95% CI [-3.6, 1.3], t(5) = -1.23, p = 0.27, d = -0.50.

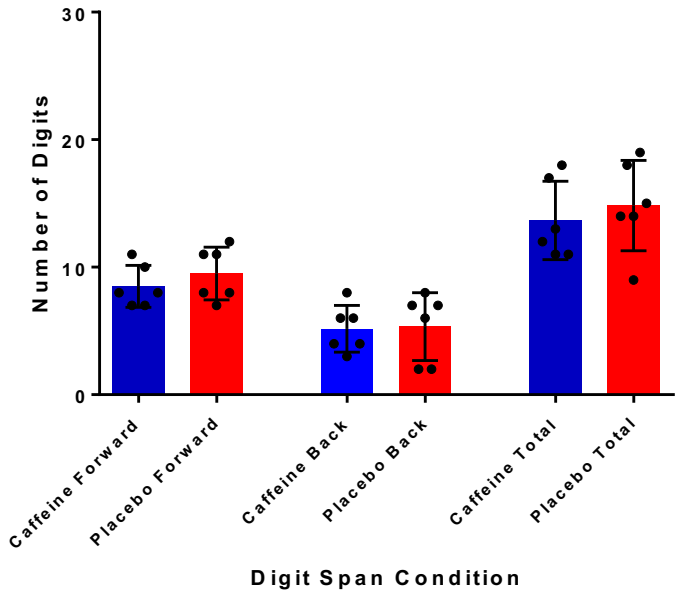

Figure 3.5b DLB digit span performance. Error bars represent standard error of the mean. No significant difference was observed.

3.3.5 Walking while talking

Healthy Elderly

A paired-samples t-test was used to determine whether there was a statistically significant mean difference between caffeine versus placebo on ***walking while talking times***. Data are mean ± standard deviation, unless otherwise stated. The assumption of normality was not violated, as assessed by Shapiro-Wilk's test for walking (p = 0.67) or walking while talking (p = 0.44) conditions.

There was no statistically significant change between walking time whilst on caffeine (12.4 s ± 2.0) compared to placebo (12.3 s ± 2.0), 1.0 s, 95% CI [-0.2, 0.4], t(17) = 0.76, p = 0.46, d = 0.18.

There was no statistically significant change between walking while talking time whilst on caffeine (12.4 s ± 2.4) compared to placebo (12.3 s ± 2.2), 0.1 s, 95% CI [-0.3, 0.5], t(17) = 0.46, p = 0.65, d = 0.11.

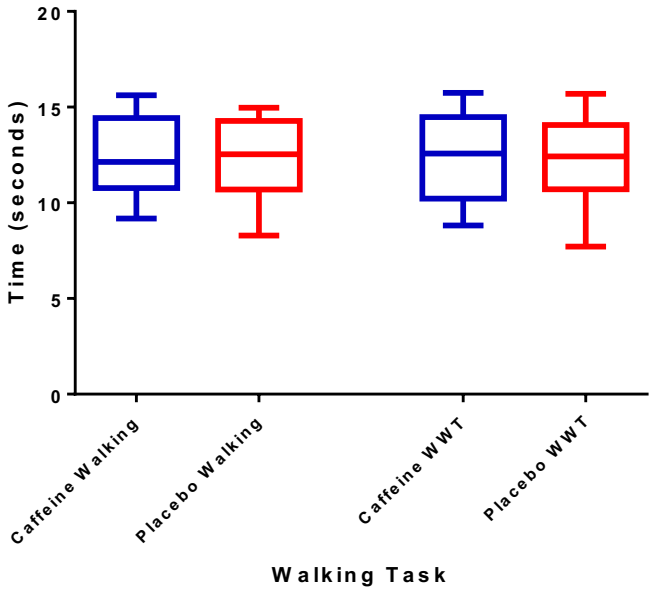

Figure 3.6a Healthy elderly walking while talking task performance. No significant difference was observed.

Dementia with Lewy bodies

A paired-samples t-test was used to determine whether there was a statistically significant mean difference between caffeine versus placebo on ***walking while talking times***. Data are mean ± standard deviation, unless otherwise stated. The assumption of normality was not violated, as assessed by Shapiro-Wilk's test for walking (p = 0.17) or walking while talking (p = 0.05) conditions.

There was no statistically significant change between walking time whilst on caffeine (22.7 s ± 5.6) compared to placebo (22.9 s ± 6.5), -0.2 s, 95% CI [-3.9, 3.5], t(5) = -0.13, p = 0.90, d = -0.05.

There was no statistically significant change between walking while talking time whilst on caffeine (22.1 s ± 4.7) compared to placebo (22.6 s ± 5.5), -0.5 s, 95% CI [-1.9, 0.8], t(5) = -1.0, p = 0.34, d = -0.43.

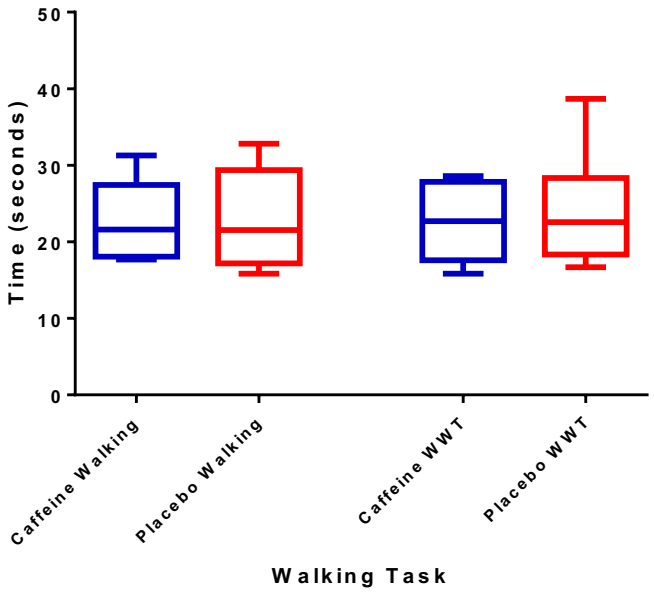

Figure 3.6b DLB walking while talking task performance. No significant difference was observed.

3.3.6 Co-variables

There was no significant correlation between age, MoCA score, sex or habitual caffeine intake and any of the tests described above.
There was no effect of intervention crossover order as a between subjects variable for any of the tests.

3.3.7 Comparison of placebo results with aged matched controls

Demographics

	DLB	Controls
Participants	6	20
Age	73.0 (67-81)	71.1 (52-87)
Sex	5 male : 1 female	6 male : 14 female
Baseline MoCA	19.17 (14-23)	27.75 (24-30)
Habitual daily caffeine intake (mg)	44 (0-85)	86 (0-170)

Table 5.2 Comparison of PD and healthy control participant demographics

3.3.8 Alerting attention

A Welch t-test was run to determine if there were differences on ***simple reaction time*** scores between DLB and aged matched healthy participants, due to the assumption of homogeneity of variances being violated, as assessed by Levene's test for equality of variances ($p = 0.03$). There were 6 DLB participants and 20 aged matched healthy participants. Data are mean ± standard deviation, unless otherwise stated. The simple reaction time scores were faster for healthy participants (307 ms ± 46) than DLB participants (4345 ms ± 95), a statistically significant difference, -128 ms, 95% CI [-228, -28], $t(5.697) = -3.19$, $p = 0.02$, $d = 1.72$

A Welch t-test was run to determine if there were differences on ***choice reaction time*** scores between DLB and aged matched healthy participants, due to the assumption of homogeneity of variances being violated, as assessed by Levene's test for equality of variances ($p < 0.01$). There were 6 DLB participants and 20 aged matched healthy participants. Data are mean ± standard deviation, unless otherwise stated. The choice reaction time scores were faster for healthy participants (525 ms

± 59) than DLB participants (868 ms ± 186), a statistically significant difference, -343 ms, 95% CI [-538, -149], t(5.307) = -4.47, p = 0.01, d = 2.49

A Welch t-test was run to determine if there were differences on ***choice reaction time*** error scores between DLB and aged matched healthy participants, due to the assumption of homogeneity of variances being violated, as assessed by Levene's test for equality of variances (p = 0.02). There were 6 DLB participants and 20 aged matched healthy participants. Data are mean ± standard deviation, unless otherwise stated. The choice reaction time error rates were fewer for healthy participants (1.4 % ± 1.1) than DLB participants (2.5 % ± 2.9), a non statistically significant difference, -1.1 %, 95% CI [-4.1, 1.9], t(5.401) = -0.92, p = 0.40, d = 0.51

A Welch t-test was run to determine if there were differences on ***cognitive reaction time*** scores between DLB and aged matched healthy participants, due to the assumption of homogeneity of variances being violated, as assessed by Levene's test for equality of variances (p < 0.01). There were 6 DLB participants and 20 aged matched healthy participants. Data are mean ± standard deviation, unless otherwise stated. The cognitive reaction time scores were faster for healthy participants (218 ms ± 51) than DLB participants (433 ms ± 183), a statistically significant difference, -215 ms, 95% CI [-407, -23], t(5.232) = -2.84, p = 0.03, d = 1.60

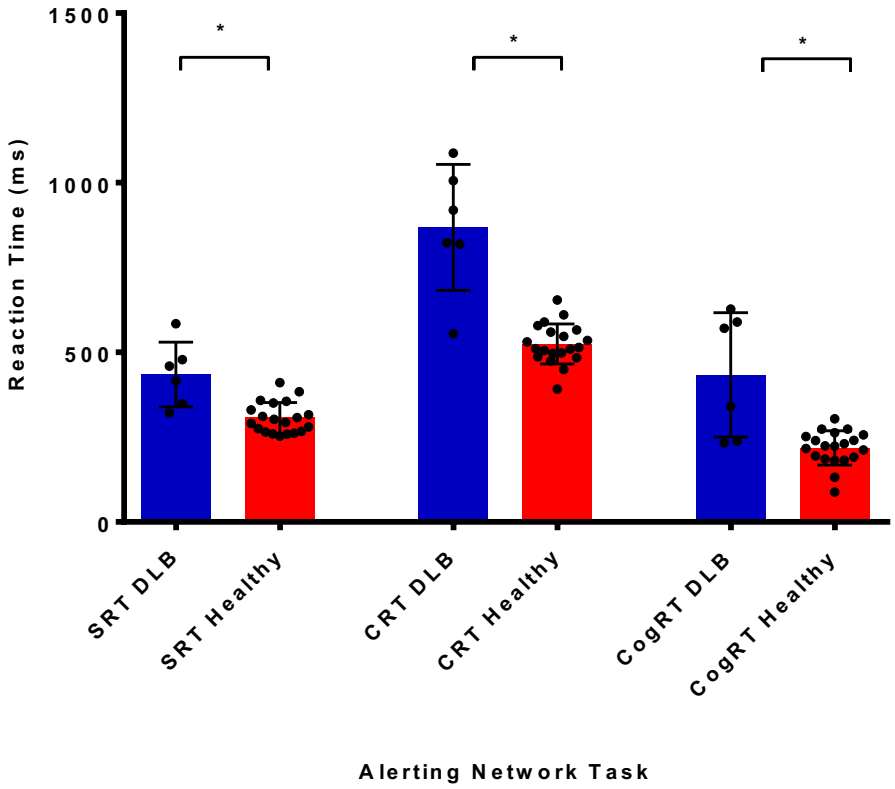

Figure 3.7 Comparing DLB and healthy elderly mean reaction time on simple reaction time (SRT), choice reaction time (CRT) and cognitive reaction time (CogRT). There was a significantly SRT, CRT and CogRT for healthy participants compared to DLB participants.

3.3.9 Orienting attention

A three-way ANOVA was run to determine the difference in accuracy between DLB and aged matched healthy participants, at different time points on the Rapid Serial Visual presentation task. There was no statistically significant three-way interaction between participant group, task and time, $F(4, 240) = 1.14$, $p = 0.34$

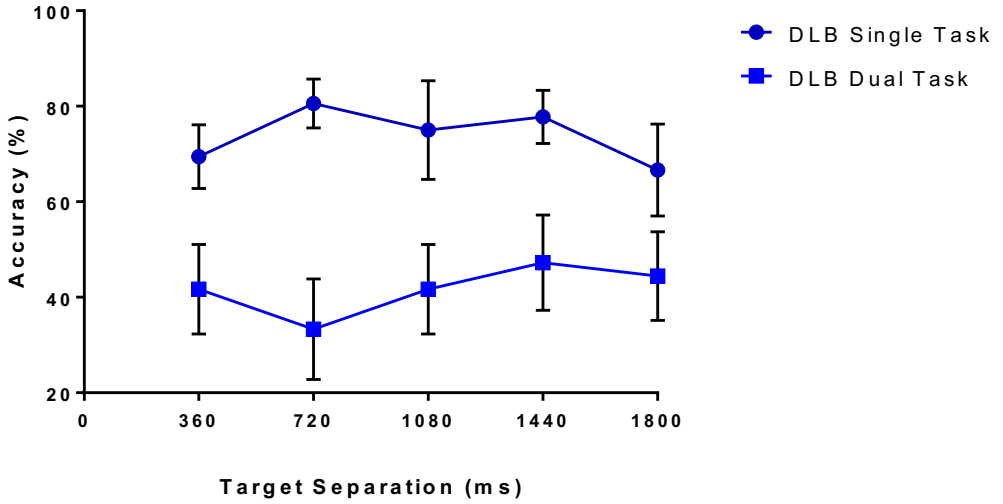

Figure 3.8a DLB mean accuracy on single and dual tasks of the Rapid Serial Visual Presentation paradigm whilst on placebo. Error bars represent standard error of the mean. The attentional blink is not observed.

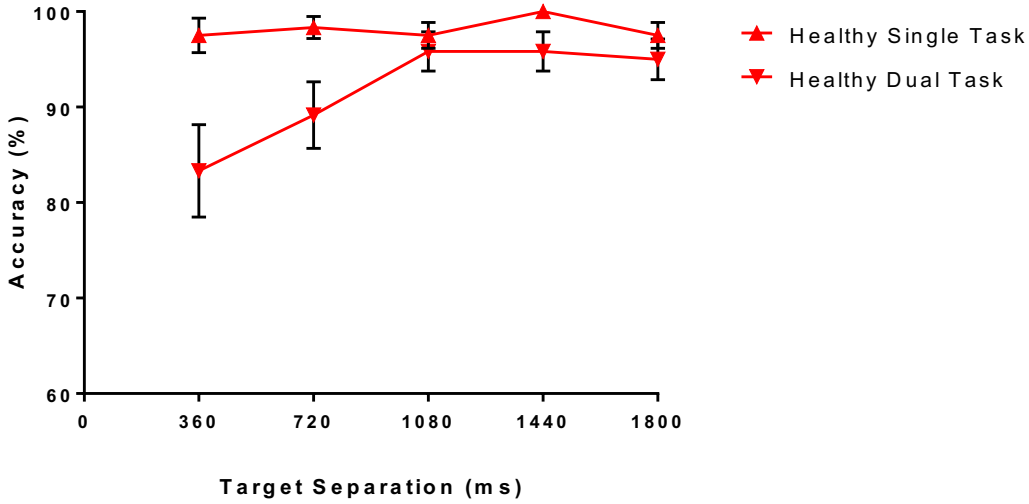

Figure 3.8b Healthy elderly mean accuracy on single and dual tasks of the Rapid Serial Visual Presentation paradigm whilst on placebo. Error bars represent standard error of the mean. The attentional blink is 1080 ms.

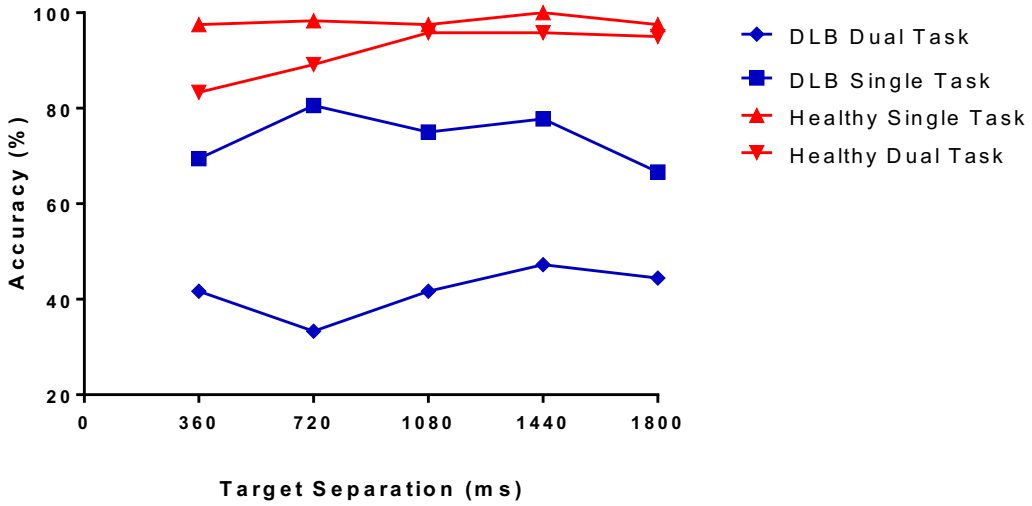

Figure 3.8c Comparing DLB and healthy elderly mean accuracy on single and dual tasks of the Rapid Serial Visual Presentation paradigm whilst on placebo.

3.3.10 Executive attention

A Welch t-test was run to determine if there were differences on ***Stroop reaction time*** scores between PD and aged matched healthy participants, due to the assumption of homogeneity of variances being violated, as assessed by Levene's test for equality of variances under neutral ($p < 0.01$), incongruent ($p < 0.01$) and total ($p < 0.01$) conditions. There were 6 DLB participants and 20 aged matched healthy participants. Data are mean ± standard deviation, unless otherwise stated. The neutral condition scores were faster for healthy participants (887 ms ± 190) than DLB participants (1964 ms ± 499), a statistically significant difference, -1077 ms, 95% CI [-1599, -554], $t(5.442) = -5.17$, $p = 0.003$, $d = 2.60$

The incongruent condition scores were faster for healthy participants (1057 ms ± 275) than DLB participants (2722 ms ± 919), a statistically significant difference, -1664 ms, 95% CI [-2627, -702], $t(5.271) = -4.38$, $p = 0.006$, $d = 2.45$

The total condition scores were faster for healthy participants (972 ms ± 229) than DLB participants (2343 ms ± 317), a statistically significant difference, -1370 ms, 95% CI [-2106, -634], $t(5.322) = -4.70$, $p = 0.01$, $d = 4.95$

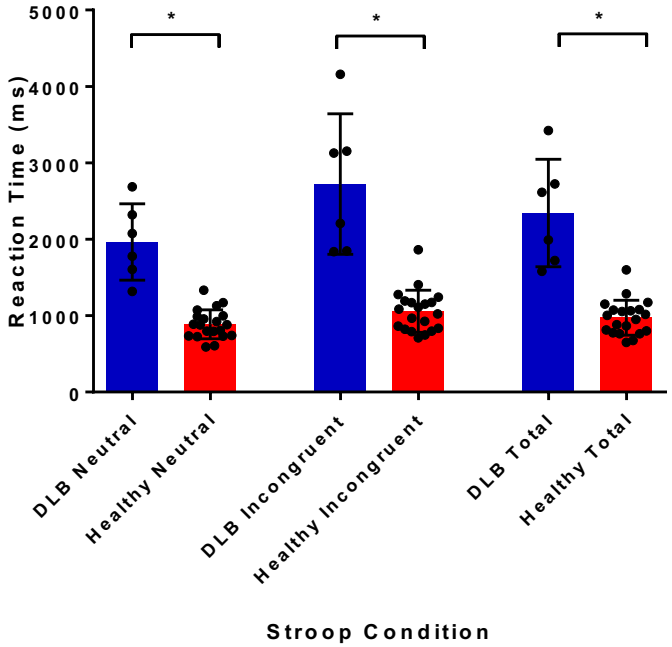

Figure 3.9 Comparing DLB and healthy elderly reaction time performance on the Stroop task. Healthy aged matched controls were significantly faster than DLB participants on all conditions.

3.3.11 Walking while talking

A Welch t-test was run to determine if there were differences on ***walking while talking*** time scores between DLB and aged matched healthy participants, due to the assumption of homogeneity of variances being violated, as assessed by Levene's test for equality of variances for walking ($p < 0.01$) and walking and walking while talking ($p = 0.01$). There were 6 DLB participants and 20 aged matched healthy participants. Data are mean ± standard deviation, unless otherwise stated.

The walking time scores were faster for healthy participants (12.3 s ± 2.0) than DLB participants (22.9 s ± 6.5), a statistically significant difference, -10.6 s, 95% CI [-17.5, -3.8], $t(53.22) = -3.91$, $p = 0.01$, $d = 2.19$

The walking while talking time scores were faster for healthy participants (12.3 s ± 2.2) than DLB participants (24.0 s ± 7.9), a statistically significant difference, -11.7 s, 95% CI [-20.0, -3.50], t(5.253) = -3.61, p = 0.01, d = 2.04

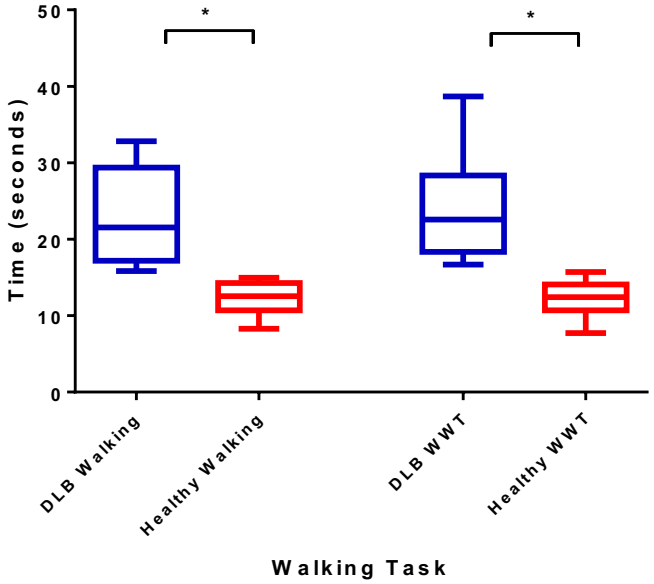

Figure 3.10 Comparing DLB and healthy elderly walking while talking task performance. DLB participants were significantly slower than aged matched controls, as expected.

3.3.12 Digit span

An independent samples t-test was run to determine if there were differences on ***digit span*** scores between DLB and aged matched healthy participants. There was homogeneity of variances, as assessed by Levene's test for equality of variances for forward (p = 0.73), backward (p = 0.86) and total (p = 0.36) conditions. There were 6 DLB participants and 20 aged matched healthy participants. Data are mean ± standard deviation, unless otherwise stated. The forward digit span scores were longer for healthy participants (11.2 ± 2.0) than DLB participants (9.5 ± 2.1), a non statistically significant difference, 1.7, 95% CI [-0.3, 3.6], t(24) = 1.75, p = 0.09, d = 0.81

The backward digit span scores were longer for healthy participants (9.9 ± 3.2) than DLB participants (5.3 ± 2.7), a statistically significant difference, 4.5, 95% CI [1.9, 7.5], $t(24) = 3.18$, $p < 0.01$, $d = 1.55$

The total digit span scores were longer for healthy participants (21.0 ± 4.8) than DLB participants (14.8 ± 3.6), a statistically significant difference, 6.2, 95% CI [1.8, 10.5], $t(24) = 3.43$, $p < 0.01$, $d = 1.36$

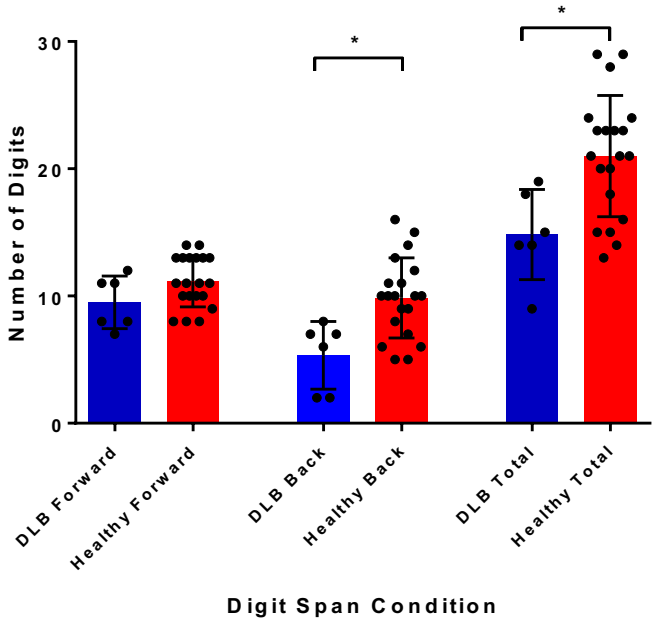

Figure 3.11 Comparing DLB and healthy elderly digit span performance. Error bars represent standard error of the mean. Healthy aged matched controls were significantly faster than DLB participants on backwards and total digit span.

3.4 Discussion

3.4.1 No effect of caffeine on attention in DLB or healthy elderly

This study investigated the effect of caffeine on each individual subtype of attention, in DLB participants and aged matched healthy controls. The original hypothesis was caffeine would improve tests of attention, specifically alerting (cognitive reaction time) and executive (Stroop reaction time) attentional domains

with no effect on orienting attention (RSVP), in both DLB and healthy elderly participants. Digit span and walking while talking task were tested to demonstrate ecological validity of any attentional enhancement.

These results *could* indicate caffeine has no significant effect on tests of attention in either DLB or aged matched healthy elderly participants. This directly contradicts my prediction, however, several significant limitations diminish the validity of these results and subsequent assertions, discussed below. Put in a real world context, it can be confidently inferred these results demonstrate acute caffeine ingestion *at a normal dietary dose* via instant coffee will not improve attention in the alerting, orienting or executive domains for healthy elderly people. It is possible a greater dose of caffeine could produce a different effect.

There is already a significant body of research examining the response of fatigue and attention to caffeine in healthy individuals. A dichotomy exists within the literature as several studies have shown an improvement in attention following caffeine ingestion (Nehlig, 2010, Einother and Giesbrecht, 2013) whilst other authors suggest this improvement is merely reversal of caffeine withdrawal (Smith et al., 2013, Rogers et al., 2013). These results support the notion that contrary to popular belief, caffeine does not improve attention and improvements demonstrated in other studies are due to withdrawal reversal. The caveat with this statement is its limitation to healthy individuals. Other stimulants such as methylphenidate, which is used to treat attention deficit hyperactive disorder, when tested in health individuals have actually produced a detrimental effect on attention (Clatworthy et al., 2009).

It is hypothesized that in people whose attention is already optimised, attentional enhancers are not beneficial and can imbalance the interplay of the attentional networks leading to impairment (Schabram et al., 2014). The same may be true for caffeine, it may improve attention but not in those whose attention is already optimal i.e. healthy controls. Most studies of caffeine have been performed

in young healthy adults where optimal attention as baseline is expected. With advancing age mild cognitive decline is expected (Ardila et al., 2000) as mild degeneration of the brain (Scahill et al., 2003a) is a normal or at least a consistent finding. It was therefore plausible that caffeine could have had a mild attention enhancing effect in this age group.

3.4.2 Comparing the attention profile of DLB to aged matched controls

Whilst not an initial aim of the study, as the design is a placebo controlled cross over, the attention characteristics of DLB can be compared directly with healthy aged matched controls. DLB performance is approximately half as good as healthy elderly controls as demonstrated by double the reaction time duration on alerting attention tasks and being just over half as accurate on the orienting attention RSVP task. As expected walking and hence walking while talking were much slower in keeping with the bradykinesia associated the DLB. The difference in digit span was more selective with proportionally a greater deficit arising with reverse digit span rather than forward digit span. This ties in with executive attention reaction times being 3-4 times longer than healthy controls.

Overall this suggests DLB patients have widespread impairments of attention which are most pronounced in the executive domain. A pathological feature of DLB is Parkinsonism related to loss of dopaminergic neurons in the substantia nigra. Cholinergic neurons and noradrenergic neurons are affected but to a lesser degree. This pattern of attention deficits adhere to Posner's model (Posner, 2012). Being able to model attention deficits accurately will allow specific enhancing therapies to be targeted according to the mode of neurotransmitter enhancement.

3.4.3 Limitations

The DLB cohort is clearly underpowered and it is therefore difficult to draw any firm conclusion from the results due to the risk of type II error. Recruitment has

been difficult with the DLB population as appropriate potential participant numbers on the research database were fewer than originally estimated.

In situations where recruitment is foreseen to be poor, data reliability can be improved by increasing the data yield per individual i.e. increasing the duration of testing. This was not a viable option as participants with cognitive impairment DLB or otherwise, suffer cognitive fatigue, which limits their ability to participate with long testing sessions. If the testing protocol had been increased it could conversely deter potential participants enrolling in the study.

Following discussion with neurologists who specialise in movement disorders, they advised me that in people already diagnosed with Parkinson's disease, an incurable neurodegenerative disease, if they develop dementia they may initiate dementia treatment but not formally give them a diagnosis. This is in case giving a diagnosis of another incurable, degenerative disease with no effective treatment causes adverse psychological effects. A future avenue for investigation would be to assess the effect of caffeine, at an appropriate dose, on attention in participants with Parkinson's disease and cognitive impairment. This will allow improved recruitment, great enough to deliver study power whilst still providing a population with impaired attention that may benefit with caffeine enhancement.

A potentially significant weakness of the study is the caffeine dose choice. The optimum dose is simply not known (Einother and Giesbrecht, 2013) and will likely vary from population to population and indeed between individuals within a population. An inverted 'U' shaped dose-response relationship has been proposed for cognitive (including attentional) enhancers that mediate their action through attenuating neurotransmitter pathways (Husain and Mehta, 2011). Therefore a caffeine dose either too high or too low may have no or even a negative effect. In this study it is possible the caffeine dose was inadequate to produce a beneficial attentional effect.

3.4.4 Conclusions

The challenge of dementia is truly upon us as highlighted by a national drive to develop treatments for this devastating syndrome. DLB is characterised by fluctuations in attention and is symptomatically treated with cholinesterase inhibitors, which induce an effect through improving cholinergic pathways in the basal forebrain and consequently improving attention. By extension, caffeine, a lauded attentional enhancer, could potentially produce a beneficial effect in people with DLB. Difficulties with recruitment severely limited participant numbers, producing underpowered data of limited value. This is unfortunate as if the trial had been successful and the data robust, a safe, cost effective treatment could be readily dispensed to DLB sufferers, improving their quality of life.

At habitual dietary doses, caffeine does not appear to improve attention in healthy elderly participants who are fully withdrawn from caffeine. This is an interesting finding and suggests one of two possibilities. Firstly, healthy elderly people may already be at their optimal attention and therefore not amenable to enhancement, in keeping with the Yerkes-Dodson law. Secondly, the dose of caffeine may not have been great enough to produce a symptomatic effect. The dose of caffeine contained in the standardised sachet of branded coffee which was used, was much less than advertised, as discussed below. This warrants further investigation as it runs counter to the preponderance of caffeine research, which supports its properties as an attentional enhancer. A future powered study with a higher caffeine dose is warranted.

3.4.5 Subsequent Protocol Amendments

The caffeine dose contained in 2g sachets of Starbucks VIA Ready Brew Italian Roast has been analysed by the University of Bristol chemistry laboratories. The normal caffeinated coffee sachet produced 63mg of caffeine whilst the decaffeinated coffee sachet produced 3mg of caffeine. This 63mg is much lower than the 135mg of caffeine that was expected to be produced. This should still be large

enough to produce a physiological effect, however, a minimum optimum dose for effect would probably be closer to 100mg. The optimum dose has in fact not been conclusively elucidated in the literature but caffeine manufacturers of over the counter supplements (Proplus) suggest a dose of 100mg. The current dose falls well below this and may be the reason for lack of observed effect. Future iterations of the protocol will use 100mg of Proplus dissolved in 2g sachets of Starbucks VIA Ready Brew decaffeinated coffee as the intervention.

The parameters for the RSVP paradigm were potentially too coarse to identify significant differences between participants or testing conditions. Specifically the interval between 'Target 1' and 'Target 2' occurred at too wide intervals, potentially missing signal data occurring in the interim. The interval durations will therefore be changed from 360 ms, 720 ms, 1080 ms, 1440 ms or 1800 ms to occur at 180 ms, 360 ms, 540 ms, 720 ms, 900 ms, 1080 ms or 1260 ms.

Chapter 4

The utility of caffeine as an attentional enhancer in healthy elderly participants

4.1 Introduction

Caffeine containing products have been marketed as a cognitive stimulant for centuries, with the first clinical trial investigating its psychological effects just over 100 years old (Benjamin et al., 1991). Despite a century of research in this field, a controversy exists within the caffeine literature as to the nature of cognitive enhancement. Most papers endorse beneficial attentional enhancement induced by acute caffeine ingestion (Nehlig, 2010, Nehlig, 2016, Haskell et al., 2005). However, another school of thought, identifies reversal of caffeine withdrawal as the likely source of positive data (James and Rogers, 2005).

There is consensus over caffeine's ability to promote wakefulness through cerebral adenosine inhibition (Burke et al., 2015). Whilst there is clearly an overlap between wakefulness and attention, these two are distinct entities with separate neuroanatomical pathways and functions. It is possible to be awake but inattentive, for example driving a car when tired (Heatherley, 2011). Likewise one can be asleep but attentive, such as waking up when presented with stimuli representing danger such as a loud noise (Rechtschaffen et al., 1966).

The caffeine withdrawal reversal theorem asserts the benefits of caffeine stem from treating withdrawal symptoms. Most clinical studies do not allow participants to withdraw for long enough before experimental testing. Caffeine withdrawal symptoms typically peak by 51 hours and subside within 5 days, but

potentially last up to 9 days (Juliano and Griffiths, 2004). Therefore any studies wishing to negate caffeine withdrawal reversal as a confounding factor should include a caffeine abstinence period as long as possible, ideally at least 1 week.

When one examines the methodology of the studies demonstrating positive attentional enhancement following caffeine, a recurring pattern is clear, the withdrawal period is consistently less than 48 hours (please see Background chapter for a detailed review). From studies employing a caffeine withdrawal period of at least 4 days, the following themes emerge: (i) caffeine may have a beneficial effect on attention in sleep deprived individuals but this has not been clearly demonstrated in non sleep deprived populations; (ii) there have been no randomised controlled trials systematically investigating the effect of caffeine on all 3 subtypes of attention, alerting, orienting and executive; (iii) there are no randomised controlled trials assessing caffeine's effect on attention in healthy elderly people.

Poorly designed studies have characterised the literature, which in association with limited critical analysis of study methodology by reviewers has propagated the narrative of caffeine as an attentional enhancer (Einother and Giesbrecht, 2013). There is consensus caffeine produces beneficial mood effects but there is no *objective* evidence, which demonstrates caffeine produces attentional enhancement in cognitively normal individuals, independent of caffeine withdrawal reversal.

4.1.1 Aims

To assess whether 100mg of caffeine compared to placebo improves attention in fully withdrawn healthy elderly participants on computerised neuropsychology paradigms and functional tasks of attention.

4.1.2 Hypothesis

Acute caffeine ingestion will improve attention in the alerting and executive domain in healthy elderly people.

4.2 Methods

4.2.1 Participants

Forty-two healthy elderly participants were tested. They were recruited from a research volunteer database held in North Bristol NHS Trusts dementia service. No healthy volunteers were diagnosed with any neurological disease.

The inclusion criteria for healthy elderly participants were:

- adequate vision to perform the tasks
- an adequate level of communication in written and verbal English
- independently mobile

The exclusion criteria for healthy participants were:

- any concomitant serious illness likely to interfere with cognitive or physical performance
- any reported cognitive problems
- signs of cognitive impairment (e.g. Montreal Cognitive Assessment <23)
- inability to consent to research, in keeping with the Mental Capacity Act 2005

Participants	42
Age	72.86 (55-91)
Sex	17 male : 25 female
Baseline MoCA	26.88 (23-30)
Habitual daily caffeine intake (mg)	105.13 (5-340)

Table 4.1 demographics of health participants

All participants had been stable on their current medication for at least three months and there were no medication changes during the trial.

Healthy Recruitment

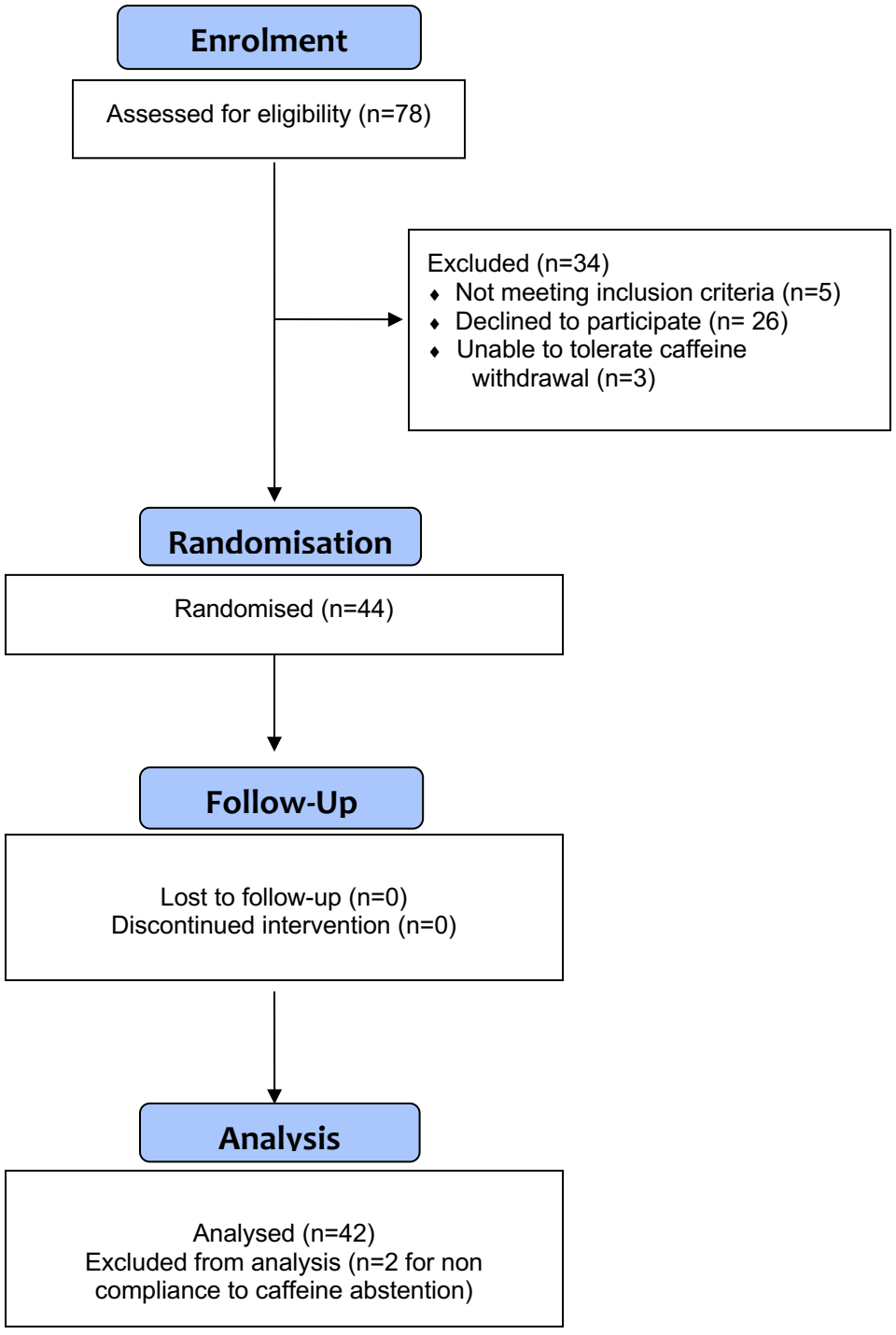

Figure 4.1 Recruitment phases for healthy elderly participants in this placebo controlled, cross over trial. Adapted from Consolidated Standards of Reporting Trials Group (Moher et al., 2001)

4.2.2 Procedure

A single blind, crossover trial compared 100mg caffeine (Proplus) tablets dissolved in instant decaffeinated coffee, with instant decaffeinated coffee. The coffee was served with or without artificial sweetener as per patient preference but consistently given across the trial. Milk was not offered. The drink was served at a temperature range of between 50 - 60ºC which was confirmed by measurement with a thermometer. This ensured the drink was hot but not too hot for safe consumption.

Participants attended for baseline testing on day 1 without any dietary caffeine restriction. Following testing they were given a supply of either decaffeinated coffee and/or decaffeinated tea to cover the trial duration (as per their consumption preference) and requested to not ingest caffeine containing foods such as tea, coffee, chocolate etc. for the remainder of the trial (9 days) but could freely consume the decaffeinated tea/coffee we supplied them. On day seven (i.e. 1 week free from caffeine) participants repeated testing to assess for effects of caffeine withdrawal on attention and allow task familiarisation so that the effect of learning on subsequent performance was minimised. On day eight participants received either caffeinated or decaffeinated coffee and testing started 60 minutes later. In the interim, participants would wait in a quiet waiting room with books and magazines for interest if desired. On day nine the participants received the alternative type of coffee (caffeinated or decaffeinated whichever not already had) and began testing 60 minutes following consumption. Testing was performed within 15 minutes of the same time on all days.

4.2.3 Task

The task battery consisted of:

i. The Montreal Cognitive Assessment (MoCA)
ii. Digit span

iii. Simple reaction time
iv. Choice reaction time
v. The rapid serial visual presentation (RSVP) paradigm
vi. Stroop task
vii. Walking while talking test (WWT)

The task battery was chosen to individually assess each of the three attentional networks as outlined in the Chapter 1. Alerting attention was assessed by measuring cognitive reaction time (choice reaction time minus simple reaction time); orienting attention was assessed by the RSVP paradigm and executive attention was assessed by Stroop task. Digit span was included as a functional test of working memory and walking while talking was included as a more ecologically valid test of attention. More detailed explanations of each of the tests are available in Chapter 2.

4.3 Results

4.3.1 Alerting attention

A paired-samples t-test was used to determine whether there was a statistically significant mean difference between caffeine versus placebo on ***simple reaction time*** scores. Data are mean ± standard deviation, unless otherwise stated. The assumption of normality was not violated, as assessed by Shapiro-Wilk's test ($p = 0.38$). There was no statistically significant change between reaction time whilst on caffeine (301 ms ± 42) compared to placebo (303 ms ± 42), -2 ms, 95% CI [-8, 4], $t(41) = -0.75$, $p = 0.46$, $d = 0.12$.

A paired-samples t-test was used to determine whether there was a statistically significant mean difference between caffeine versus placebo on ***choice reaction time*** scores. Data are mean ± standard deviation, unless otherwise stated. The assumption of normality was not violated, as assessed by Shapiro-Wilk's test (p

= 0.11). There was no statistically significant change between reaction time whilst on caffeine (514 ms ± 65) compared to placebo (524 ms ± 66), -10 ms, 95% CI [-201, 0], t(43) = -1.99, p = 0.054, d = 0.31.

A sign test with continuity correction was used determine whether there was a statistically significant median difference between caffeine versus placebo on ***choice reaction time*** error rates. Of the 42 participants recruited to the study, caffeine ingestion compared to placebo reduced errors in 11 participants, increased errors in 18 participants and had no effect on 13 participants. There was no statistically significant difference between errors whilst on caffeine (Median = 0.7) compared to placebo (Median = 1.0), -0.33, z = 1.11, p = 0.27.

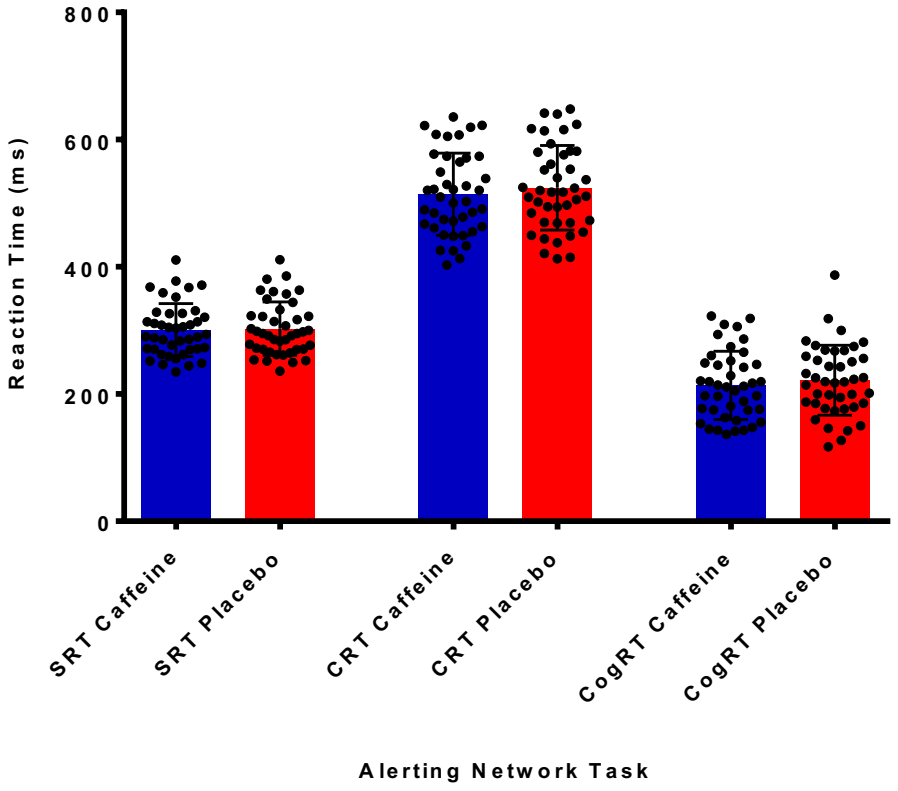

Figure 4.2 Healthy elderly mean reaction time on simple reaction time (SRT), choice reaction time (CRT) and cognitive reaction time (CogRT).

A paired-samples t-test was used to determine whether there was a statistically significant mean difference between caffeine versus placebo on ***cognitive reaction time*** scores. Data are mean ± standard deviation, unless otherwise stated. The assumption of normality was not violated, as assessed by Shapiro-Wilk's test (p = 0.85). There was no statistically significant change between reaction time whilst on caffeine (214 ms ± 54) compared to placebo (222 ms ± 55), -8 ms, 95% CI [-19, 30], t(41) = -1.47, p = 0.15, d = 0.23.

4.3.2 Orienting attention

A three-way repeated measures ANOVA was run to determine the effect of caffeine on accuracy at different time points on the Rapid Serial Visual presentation task. Mauchly's test of sphericity indicated the assumption of sphericity was met for the three-way interaction, $\chi 2(40) = 30.75$, p = 0.06. There was no statistically significant three-way interaction between caffeine, task and time, F(6, 246) = 1.23, p = 0.29.

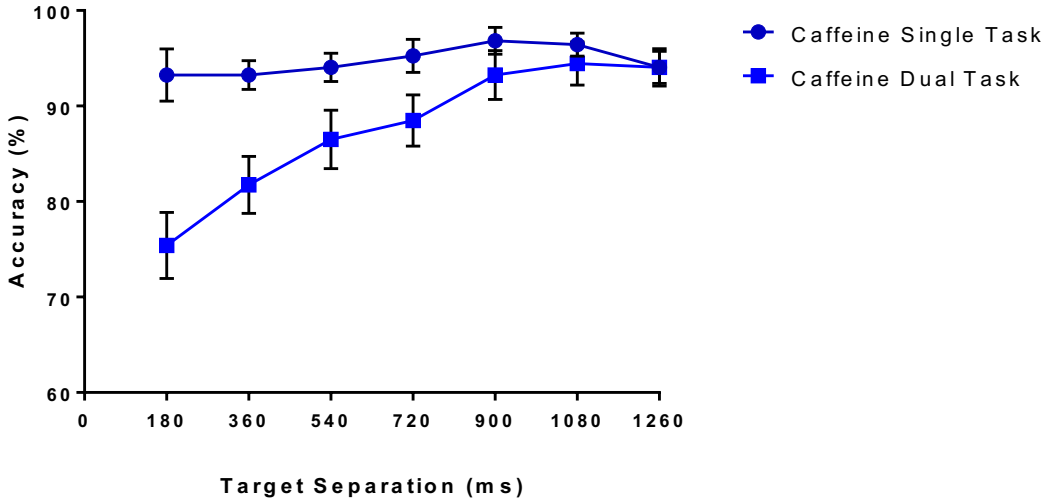

Figure 4.3a Healthy elderly mean accuracy on single and dual tasks of the Rapid Serial Visual Presentation paradigm whilst on caffeine. Error bars represent standard error of the mean.

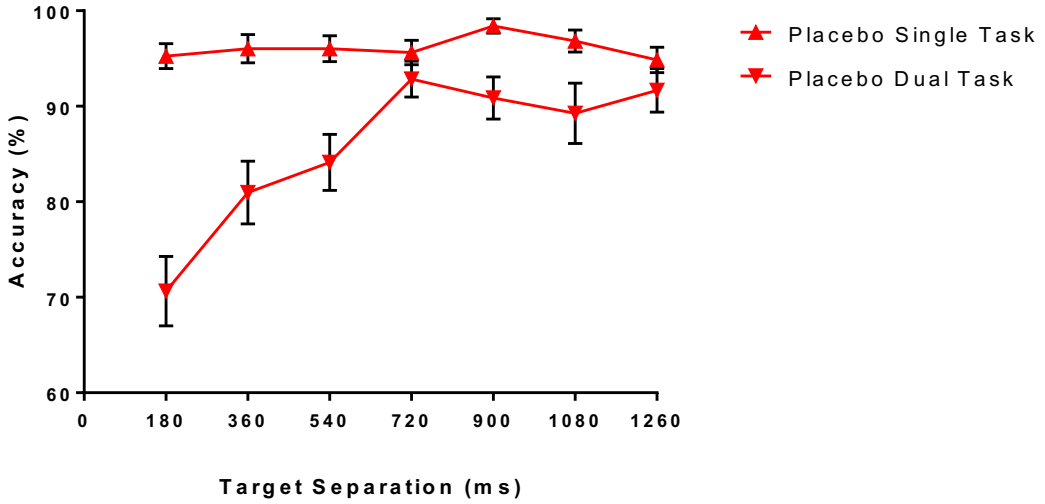

Figure 4.3b Healthy elderly mean accuracy on single and dual tasks of the Rapid Serial Visual Presentation paradigm whilst on placebo. Error bars represent standard error of the mean.

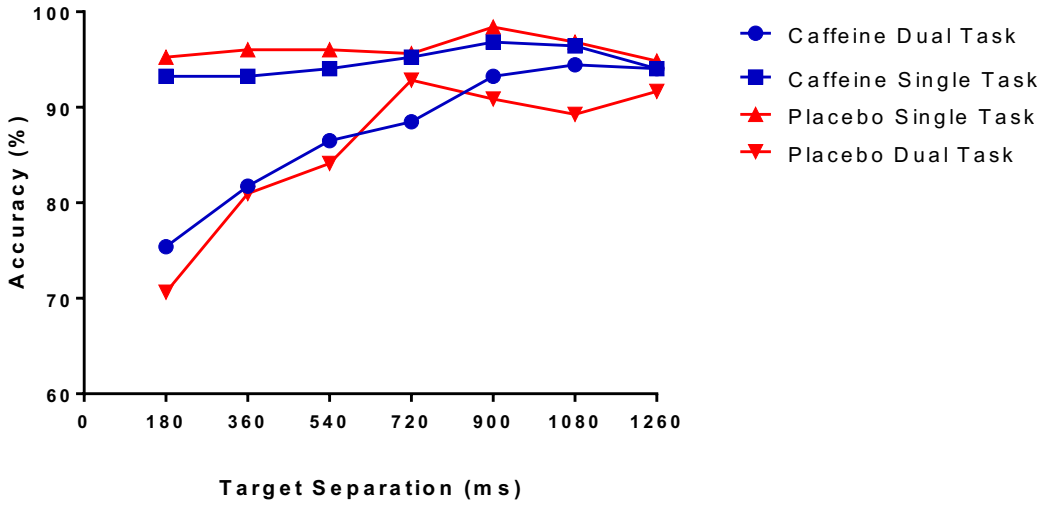

Figure 4.3c Healthy elderly mean accuracy on single and dual tasks of the Rapid Serial Visual Presentation paradigm comparing caffeine to placebo. Error bars represent standard error of the mean.

4.3.3 Executive attention

A paired-samples t-test was used to determine whether there was a statistically significant mean difference between caffeine versus placebo on ***Stroop reaction time*** scores. Data are mean ± standard deviation, unless otherwise stated. The assumption of normality was not violated, as assessed by Shapiro-Wilk's test for

neutral (p = 0.15) and total (p = 0.15) conditions, however, the incongruent (p = 0.01) condition was not normally distributed.

There was no statistically significant change in reaction time during the neutral condition whilst on caffeine (851 ms ± 128) compared to placebo (853 ms ± 129), -2 ms, 95% CI [-23, 18], t(41) = -0.24, p = 0.81, d = 0.04.

There was no statistically significant change in total Stroop reaction time whilst on caffeine (914 ms ± 150) compared to placebo (917 ms ± 149), -3 ms, 95% CI [-27.045, 20.136], t(41) = -0.296, p = -0.769, d = 0.05.

A sign test with continuity correction was used determine whether there was a statistically significant median difference between caffeine versus placebo on incongruent condition reaction time on the Stroop task. Of the 42 participants recruited to the study, caffeine ingestion compared to placebo reduced (quickened) reaction time in 22 participants and increased reaction time in 20 participants. There was no statistically significant difference between errors whilst on caffeine (Median = 965) compared to placebo (Median = 982), -17, z = 0.15, p = 0.88.

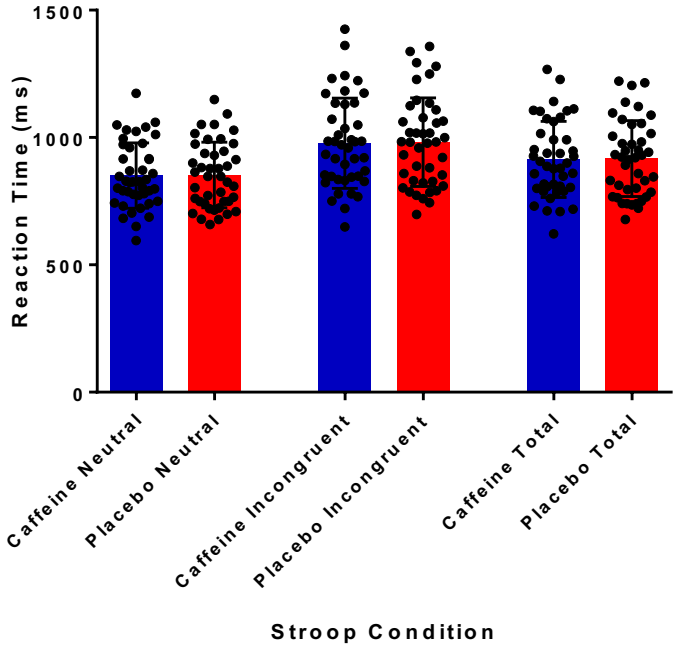

Figure 4.4 Healthy elderly reaction time performance on the Stroop task.

4.3.4 Digit Span

A paired-samples t-test was used to determine whether there was a statistically significant mean difference between caffeine versus placebo on ***digit span score***s. Data are mean ± standard deviation, unless otherwise stated. The assumption of normality was not violated, as assessed by Shapiro-Wilk's test for forward ($p = 0.09$) and total ($p = 0.22$) conditions, however, backwards ($p = 0.03$ digit span was not normally distributed.

There was no statistically significant change in digit span during the forward condition whilst on caffeine (10.8 digits ± 2.4) compared to placebo (10.9 digits ± 2.2), -0.1 digits, 95% CI [-0.6, 0.5], $t(41) = -0.4$, $p = 0.73$, $d = 0.05$.

There was no statistically significant change in total digit span whilst on caffeine (18.4 digits ± 4.0) compared to placebo (18.7 digits ± 3.8), -0.3 digits, 95% CI [-0.9, 0.4], $t(41) = -0.8$, $p = 0.44$, $d = 0.12$.

A Wilcoxon signed-rank test was used determine whether there was a statistically significant median difference between caffeine versus placebo on backwards digit span. The difference scores were symmetrically distributed, as assessed by a histogram. Of the 42 participants recruited to the study, caffeine increased digit span in 12 participants, decreased digit span in 19 participants and had no effect in 11 participants. There was no statistically significant difference between backwards digit span whilst on caffeine (Median = 7) compared to placebo (Median = 7), $z = 0.60$, $p = 0.88$.

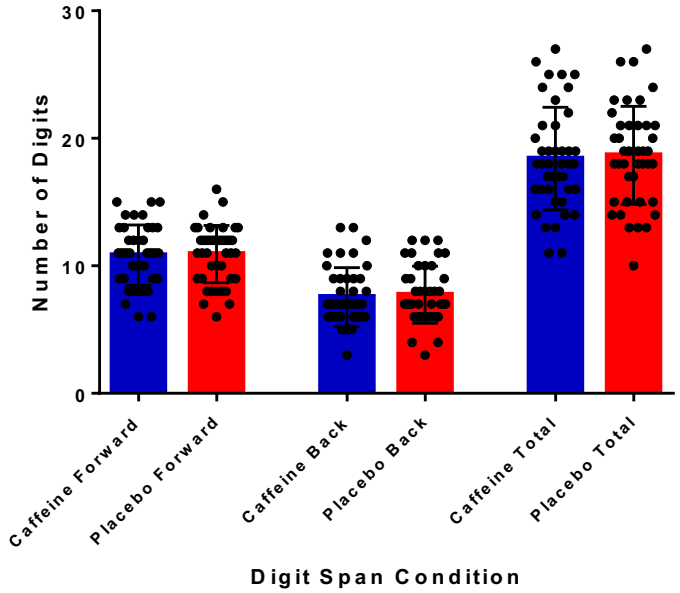

Figure 4.5 Healthy elderly digit span performance. Error bars represent standard error of the mean.

4.3.5 Walking while talking

A related samples Sign test with continuity correction was used to determine whether there was a statistically significant median difference between caffeine versus placebo on *walking while talking* times. Of the 42 participants recruited to the study, caffeine ingestion compared to placebo reduced (quickened) walking time in 20 participants and increased walking time in 22 participants. There was no statistically significant difference in walking times whilst on caffeine (Median = 14.6) compared to placebo (Median = 14.7), -0.2, z = 0.15, p = 0.88.

Of the 42 participants recruited to the study, caffeine ingestion compared to placebo reduced (quickened) walking while talking time in 19 participants and increased walking while talking time in 23 participants. There was no statistically significant difference in walking while talking times whilst on caffeine (Median = 14.6) compared to placebo (Median = 15.0), -0.4, z = -0.46, p = 0.64.

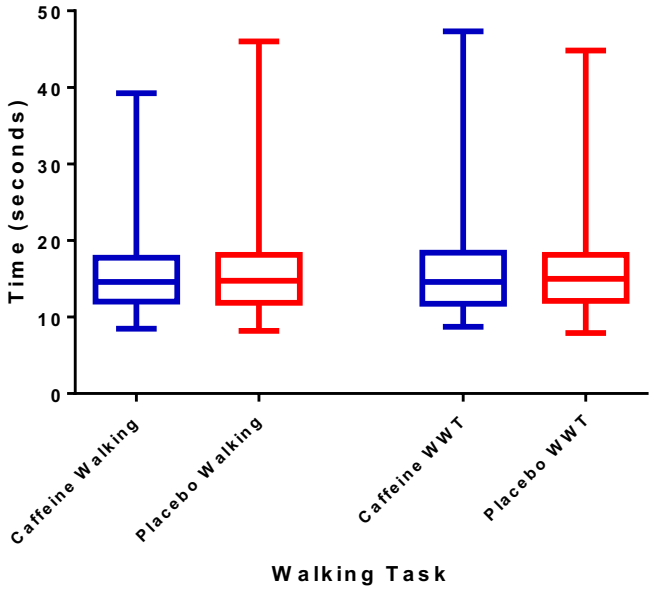

Figure 4.6 Healthy elderly walking while talking task performance

4.3.6 Correlations

A Pearson product-moment correlation coefficient was run to assess the relationship between simple reaction time and baseline caffeine ingestion. There was a moderate positive correlation between simple reaction time and baseline caffeine ingestion $r(40) = 0.41$, $p = 0.01$. The higher the baseline caffeine levels the longer the simple reaction time scores.

A Pearson product-moment correlation coefficient was run to assess the relationship between choice reaction time and MoCA score. There was a moderate negative correlation between choice reaction time and MoCA score $r(40) = -0.51$, $p = 0.001$. The higher the MoCA score the shorter the choice reaction time score.

There was no other significant correlation between age, MoCA score, sex or habitual caffeine intake and any of the tests described above.
There was no effect of intervention crossover order as a between subjects variable for any of the tests.

4.3.7 Post hoc power analyses

To assess whether the non-significant results were the result of type II error, I conducted post hoc power analyses using G*Power (Faul et al., 2007) with power (1 - β) set at 0.80 and α = 0.05, two-tailed.

To test my hypothesis with statistical significance at the 0.05 level:

- alerting attention sample sizes would be N = 547, 84 and 151 for SRT, CRT and CogRT, respectively
- orienting attention sample sizes would be N = 42
- executive attention sample sizes would be N = 4908, 3503 and 3142 for congruent, incongruent and average Stroop time, respectively
- digit span sample sizes would be N = 3142, 654 and 547 for forward, backward and total digit span, respectively
- WWT sample sizes would be N = 13740 and 10 for walking and WWT, respectively

4.4 Discussion

4.4.1 Caffeine does not enhance attention in healthy elderly participants

This study investigated the effect of caffeine on each individual subtype of attention in healthy elderly participants. In direct contradiction to the original hypothesis, caffeine did not significant improve performance on tasks testing any attentional network, real-work task of attention or functional tasks of working memory. This is surprising given the abundance of published studies with results to the contrary, I will explore the reasons behind this.

Only a handful of published randomised controlled trials test the acute attentional effects of caffeine following a withdrawal period of 4 days or longer (Kamimori et al., 2015, Smith et al., 2013, Rogers et al., 2005, Judelson et al., 2005) whilst the remaining 95% of published trials typically use a withdrawal period of less than 48 hours. This study's findings are supportive of the *caffeine withdrawal reversal hypothesis*, which asserts positive effects of caffeine demonstrated in trials in which participants are inadequately withdrawn, are not due to a net improvement in attention but instead indicate the reversal of the fatiguing effects of withdrawal.

The issue of whether caffeine improves attention above baseline once participants have been fully withdrawn, has not been clearly elucidated in previous papers. Here we employed a more rigorous testing procedure to ensure adequate time for participants to be fully withdrawn from caffeine before being randomised to caffeine or placebo whilst also systematically assessing its effects on the trinity of attentional networks. Whilst this negative study is in the minority, due to rigor it holds greater weight than most published work, which by comparison is arguably less robust.

Caffeine mediates its effect through cerebral adenosine receptor antagonism, which promotes wakefulness. It inhibits the effect of GABA neurons and promotes the release of norepinephrine, dopamine and acetylcholine. Therefore in theory it could enhance attention as well as delay sleep. However, I did not record sleep and therefore cannot confirm or refute if caffeine would have had a selective effect on sleep deprived individuals.

Studies investigating caffeine's pharmacological properties have been conducted in animals or young adults, whether the same pharmacological effects are exhibited in elderly people has so far not been investigated. Ageing is known to have deleterious effects on the body, which will eventually lead to death even in the absence of significant disease. With age cerebral volumes diminish, reaction times

slow and neurotransmitter pathways function with less efficiency. It is unclear if an ageing brain responds to stimulant therapy in the same way as a younger adult brain. Clinical trial data assessing the effect of stimulants and depressants in elderly populations have found participants more sensitive to side effects at start doses, suggesting that as the brain atrophies with age, the drug dose required to produce a psychomotor effect is reduced (Swift et al., 1985a) (Kumar, 2008).

A potential scepticism of the negative caffeine effect could be the dose administered. A standard dose of 100mg was chosen as is in keeping with manufacturer guidelines and licensed by the UK Medicines and Healthcare Products Regulatory Agency. This dose has been demonstrated to be safe and large enough to demonstrate a positive effect (Smit and Rogers, 2000, Lieberman et al., 1987, Hewlett and Smith, 2007) however, these studies had an inadequately short caffeine withdrawal period and therefore likely indicate only a small dose of caffeine is required to reverse withdrawal. In fully withdrawn participants or caffeine naive users, there are no phase II clinical trials assessing the optimal dosage of caffeine to induce a beneficial mood or psychomotor effect. This is an area of future research worth exploring if further clinical trials are to demonstrate validity. One could consider increasing the dose within safe limits but adverse effects can begin at 6.5mg/kg (approx. 400mg) over a day (Nawrot et al., 2003, Smith, 2002).

In the context of arousal, performance ability is proposed to follow an inverted U-shaped curve (Anderson, 1994). Optimum performance does not occur when arousal is maximal, when a state of hypervigilance ensues but is attained at a sweet spot of intermediate arousal. When we relate this back to the physiology of attention, the rational of the inverted U-shaped curve becomes apparent. Attentional processing acts as a sieve, to selectively process information relevant to goal related behaviour. If arousal is increased to the point of inducing hypervigilance, it will overload one's finite cognitive resource with stimuli irrelevant to the task at hand, thereby impairing performance directed towards the task. This has been exemplified in clinical trials using stimulants such as modafinil

(Wesensten et al., 2002). The inverted U-shaped curve of attentional performance implies impaired attention would be an appropriate target for caffeine and other stimulants. This proposal is reinforced by caffeine improving attention when ingested following sleep deprivation (Kamimori et al., 2015), although this was in a young adult population.

One may deduce that the negative effect of caffeine on attention in healthy elderly individuals without any neurological disease is due to baseline arousal and attention already in the optimum range. It is therefore probable no type of cognitive enhancement whether caffeine or otherwise could improve attentional processing in this population. It could be argued healthy elderly participants have impaired attention when compared to healthy younger participants as they perform mildly worse on the same tests of attention. However, the relatively impaired performance by healthy elderly participants represents normal aging (Fortenbaugh et al., 2015), associated with a mild generalised decrease in brain volume which occurs as a part of normal aging (Scahill et al., 2003b). In effect, healthy elderly participants already have optimum arousal/attention and produce an optimum performance but their optimum performance is never as good as the optimum performance of young healthy participants.

4.4.2 Limitations

The protocol update between Chapter 3 and the current chapter included an increase in the caffeine dose, as the initial dose was considered too low to reliably cause an effect. As the optimum dose of caffeine to produce attentional enhancement is unknown, it is possible the negative result is due to inappropriate dosing. Interestingly, the older an individual the lower the dose of caffeine required to induce a therapeutic effect (Swift and Tiplady, 1988a) despite no significant age related differences in caffeine pharmacokinetics (Blanchard and Sawers, 1983).

As with any participant recruited trial, selection bias is always an important consideration. Whilst the screening to participation rate was high for a clinical trial, a concerning issue was the inability of two participants to maintain caffeine abstinence during the trial. Caffeine abstinence was assessed through self reporting and completion of a caffeine consumption questionnaire. In many ways the drop out ratio is reassuring as this is an expected phenomenon echoed in other studies (Rogers et al., 2005). Rogers' study collected saliva from participants for caffeine level analysis, unfortunately this facility was unavailable to me and therefore I had to trust that participants had accurately completed their caffeine consumption questionnaires. The risk is undeclared caffeine consumption by participants, which could skew the results to incorrectly demonstrate a negative effect.

I intentionally excluded mood testing in the trial as I wished to focus on objective rather than subjective improvements in attention. However, in light of an unexpected negative result, it would be interesting to ascertain whether there is a disparity between subjective feelings of increased attention or arousal and a lack of objective improvement in attention. This would have required pre and post intervention mood assessment. If the disparity was confirmed it could suggest caffeine induces a feeling of reward or a sense of euphoria which make the individual *feel* more energetic and alert but without any demonstrable improvement in attentional performance.

4.4.3 Conclusion

A body of research spanning over a century has championed caffeine as a panacea for impaired attention. Fundamental study design flaws, primarily the absence of an adequate caffeine withdrawal period prior to testing, has perpetuated throughout the literature. Critics who have contested this design flaw, have received little or inadequate consideration by reviewer authors (Einother and Giesbrecht, 2013, Nehlig, 2010).

In contrast to the majority of published data, this study (i) employed an appropriate caffeine withdrawal period of 1 week prior to testing and (ii) demonstrated no improvement in attention following acute caffeine administration in healthy elderly participants. This is a novel observation, as there are no published trials investigating attentional enhancement by caffeine in healthy elderly participants, with an adequate caffeine withdrawal period.

This negative study is in keeping with the inverted U-shaped curve of arousal in relation to performance. When attention is already optimised, as is the case in non sleep deprived healthy individuals, it cannot be pharmacologically enhanced. Future studies will repeat the experimental paradigm in participants with neurological conditions such as Parkinson's disease and multiple sclerosis, where attention is impaired. Further studies are warranted in assessing the effect of caffeine on attention in healthy participants who are sleep deprived.

Chapter 5

The utility of caffeine as an attentional enhancer in Parkinson's disease

5.1 Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder, after Alzheimer's disease, affecting people aged over 65 years (Tanner and Goldman, 1996). With an ageing population the prevalence will only increase as time continues (Savica et al., 2018). Diagnosis is based on the UK Brain Bank criteria, requiring bradykinesia with at least one of rigidity, rest tremor or postural instability (Hughes et al., 1992). Non-motor features do not form part of the diagnostic classification but are well recognised and may predate the typical motor features. They typically include rapid eye movement sleep disorder, depression, constipation and impaired olfaction. Increasingly, it is being recognised that non motor symptoms correlate with worsening of health related quality of life measures, even more so than motor symptoms (Müller et al., 2013, Hely et al., 2005, Schrag et al., 2000).

The pathological hallmark of PD are Lewy bodies, which consist of fibrillar aggregates of alpha-synuclein, a protein vital for presynaptic vesicle formation. These are found in the cytoplasm of neuronal cells distributed throughout the cerebrum, however, these preferentially accumulate in monoaminergic neurons, especially the substantia nigra resulting in a loss of dopamine production (Lee and Trojanowski, 2006, Braak et al., 2006). The underlying aetiology of Lewy bodies has not been elucidated but as with other dementia substrates such as tau and amyloid, their formation is proposed as a consequence of dysfunctional protein degradation and clearance (Olanow and McNaught, 2006).

Cognitive impairment correlates with the distribution of Lewy bodies within the cerebrum. Crucially the nucleus basalis of Meynert is a frequently affected area, which synthesises acetylcholine and projects extensively to the cortex. Cholinesterase inhibitors, which decrease the breakdown of acetylcholine, have been shown to improve cognitive function and overall wellbeing in people with PD and cognitive impairment (Rolinski et al., 2012). In keeping with this principle, the Scottish Intercollegiate Guidelines Network advises against the use of anticholinergic drugs in PD due to the high risk of an adverse effect on cognition (Grosset et al., 2010).

There is a high prevalence of cognitive impairment in PD with mild cognitive impairment affecting 19% to 55% (Goldman and Litvan, 2011). This is associated with decreased quality of life, increased functional impairment and increased care needs (Martin et al., 2008, Marras et al., 2008). Initially the Mini Mental State Exam (MMSE) was proposed as a screen for cognitive impairment in PD, however, this test emphasises orientation and language which are relatively preserved early on (Emre et al., 2007a) rendering the test relatively insensitive (Isella et al., 2014). Despite normal MMSE scores, on more detailed neuropsychometry, PD sufferers commonly perform 1.5 standard deviations below the age-matched mean on tests of processing speed and learning/memory (Burdick et al., 2014). The Montreal Cognitive Assessment has been demonstrated as a more sensitive screening tool for cognitive impairment in PD (Hu et al., 2014).

Epidemiological studies have consistently demonstrated a lower risk of PD with the consumption of caffeinated drinks although it is unclear if caffeine is truly neuro protective or this represents inherent design bias. The risk of PD is 30% lower in coffee drinkers than in non-coffee drinkers (Hernan et al., 2002). Although this could reflect a neuroprotective effect of caffeine, another perspective is PD sufferers do not obtain the same health benefits from caffeine as aged match controls and therefore consume lower quantities.

Caffeine mediates its effect through up regulating several neurotransmitter networks including dopamine, which amongst other functions already described above, is also involved in mesolimbic reward mechanisms. If intrinsic dopamine production is lowered, as in PD, then ingesting caffeinating products will not induce the same positive reinforcing effect and therefore recurrent caffeine consumption behaviour is less likely. It would be interesting to assess mesolimbic dysfunction such as depression or anxiety, to assess whether there was a negative correlation with caffeine consumption.

An open label dose escalation pilot study found caffeine mildly improved PD motor manifestations (Altman et al., 2011), it also demonstrated an improvement in gait akinesia in PD sufferers with gait freezing (Kitagawa et al., 2007). A 6 week randomised controlled trial using 200-400 mg of caffeine a day, failed to show a benefit of caffeine on excessive daytime somnolence as its primary outcome but did show a beneficial effect on motor manifestations as assessed by the Unified Parkinson's Disease Rating Scale (Postuma et al., 2012). The same research group performed a similar study but over a prolonged treatment time of 6 to 18 months (Postuma et al., 2017). This demonstrated no improvement in PD motor symptoms, their primary outcome. Secondary outcomes showed a mild improvement in subjective daytime somnolence scores.

The authors suggest the unexpected lack of effect on motor symptoms and somnolence can be attributed to the theory of 'reverse causality' when considering the associations demonstrated in the epidemiological data, where caffeine non-use is associated with increased PD risk. The relationship can be explained by PD sufferers losing the beneficial effect of caffeine on attention and therefore spontaneously discontinuing its consumption; as opposed to the conventional belief of caffeine reducing the risk of PD development (Postuma et al., 2012). A significant confounding factor in this study is the absence of caffeine withdrawal and all participants were allowed to continue their habitual intake. Caffeine consumption has been proposed to be self titrated according to effect, it is likely participants were

already ingesting an optimum caffeine dose and therefore a beneficial effect would not be expected with further dose amplification.

Excessive daytime somnolence is a common, disabling, non-motor feature of PD and can be due to the disease itself or as a result of dopaminergic medication (Verbaan et al., 2008, Razmy et al., 2004). It is associated with cognitive deficits in attention, memory and executive function (Adler and Thorpy, 2005). The frequency increases with disease severity and duration (Tan et al., 2002, O'suilleabhain and Dewey Jr, 2002) affecting up to half of all PD sufferers (Abbott et al., 2005). Modafinil has been trialled as an antidote to excessive daytime somnolence with equivocal results in PD (Ondo et al., 2005, Adler et al., 2003, Högl et al., 2002). Its use across multiple diseases associated with somnolence or impaired attention is limited by the lack of understanding of its mechanism of action.

In Parkinson's disease compared to aged matched health controls, there is a decrease in A2A receptors in the dorsal striatum (caudate nucleus and putamen) but an increase in the substantia nigra pars reticulata, with no change in any other brain regions (Hurley et al., 2000). Adenosine A2A receptors are co-localised with dopaminergic D2 receptors on GABAergic neurons and have antagonising effects (Benarroch, 2008, Fredholm and Svenningsson, 2003, Ferre et al., 2008). Striatal D2 receptor activation forms part of the striatopallidal indirect pathway which is concerned with suppressing motor activity, in balance with the direct pathway in enhancing voluntary motor actions (Svenningsson et al., 1999). Adenosine A2A receptor activation theoretically suppresses GABAergic neuronal inhibition of the indirect pathway, and should therefore improve movement in PD by restoring some balance between the direct and indirect dopamine pathways (Mori et al., 1996), although this has not been conclusively demonstrated in clinical trials, discussed below. A2A receptor antagonists such as caffeine should exert a similar effect to dopamine agonists and could function as an add on to conventional levodopa therapy in PD (Vuorimaa et al., 2017).

Dopamine has been proposed to promote wakefulness by stimulating the activity of the locus coeruleus and raphe nucleus via dopaminergic D2 receptor activation (Silkis, 2009), both D1 and D2 receptor subtypes are distributed in this region. Interestingly in animal studies D2 dopamine-like agonists in contrast to dopamine, reduce wakefulness (Crochet and Sakai, 2003). An alternative explanation is dopaminergic mesocortical pathways mainly exert an effect over the frontal lobes especially the pre-frontal cortex, which plays a role in selective attention. Dopamine deficiency such as in PD will cause somnolence whilst over activity in conditions like attention deficit hyperactivity disorder, will cause loss of attentional control (Ohno, 2003). Adenosine activation could potentially promote somnolence by opposing dopamine D2 receptor activation. Caffeine, a safe and ubiquitous drug, should theoretically be an ideal wakefulness promoting medication in this situation, as it antagonises adenosine receptors and will consequentially inhibit the somnolent effect of adenosine A2A receptor activation on dopamine D2 receptor mesocortical pathways i.e. caffeine will inhibit an adenosine mediated inhibitory pathway.

Gait instability is a core feature of PD and is associated with decreased stride length and loss of gait automaticity resulting in increased falls (Blin et al., 1990, Hausdorff et al., 1998). Recurrent falls affect up to 40% of the PD population whilst 70% will fall at least once in a year (Allen et al., 2013). Gait instability not only reflects deterioration in motor function but also signifies a degree of executive dysfunction and dyspraxia. This can be attributed to cholinergic loss, impairing attention and reducing the available cognitive resource for automated activities such as walking (Rochester et al., 2004) and diverting them to concurrent goals such as talking, when performing the walking while talking task (see Methods chapter for more information)(Bloem et al., 2006). Rivastigmine, a cholinesterase inhibitor, reduces the risk of falls which may be the due to improved attentional capacity allowing more complex walking behaviours to be successfully undertaken (Henderson et al., 2016).

Functional MRI during a "dual task" walking test of PD participants demonstrated increased activity bilaterally in the dorsolateral and ventrolateral prefrontal cortices, posterior parietal regions, pre-supplementary motor areas and motor cortex, in participants who experienced freezing of gait. This cortical network has been proposed to reflect a compensatory mechanism due to the failure of basal ganglia circuitry (Shine et al., 2011). In essence advanced PD sufferers have altered brain activation patterns when walking with widespread cortical involvement, which potentially render them more susceptible to gait abnormalities when competing pathways require concurrent processing (Gilat et al., 2015).

For decades caffeine has been associated with a reduced risk of PD although a causative mechanism remains elusive. Initial trials assessing for a beneficial effect of chronic caffeine ingestion on PD motor scores and somnolence have been disappointing, however, these studies have been confounded by a lack of caffeine withdrawal in the placebo group. It remains unknown if acute caffeine ingestion in PD sufferers will improve attention. If successful it would provide a safe, cheap symptomatic treatment not only for disabling cognitive symptoms such as somnolence and slow processing but also for complex motor activities such as gait stability which is reliant on effective attentional function.

5.1.1 Aims

To assess whether 100mg of caffeine compared to placebo improves attention in fully withdrawn PD participants with cognitive impairment on computerised neuropsychology paradigms and functional tasks of attention.

5.1.2 Hypothesis

1. Acute caffeine ingestion will improve attention in the alerting and executive domain in people with PD.

2. Healthy aged matched participants (from chapter 4) will perform better than PD participants on tests of alerting and executive attention, walking and walking while talking tasks.

5.2 Methods

5.2.1 Participants

Twenty-four PD participants were recruited from a clinical research database held in North Bristol NHS Trusts.

The inclusion criteria for PD participants were:

- an established diagnosis of PD
- subjective or objective cognitive impairment, including mental fatigue
- adequate vision to perform the tasks
- an adequate level of communication in written and verbal English
- independently mobile

The exclusion criteria for PD participants were:

- any concomitant serious illness likely to interfere with cognitive or physical performance
- inability to consent to research, in keeping with the Mental Capacity Act 2005
- loss of capacity to consent to research during the trial

	PD	Controls
Participants	24	42
Age	68 (57-78)	72.9 (55-91)
Sex	15 male : 9 female	17 male : 25 female
Baseline MoCA	26.42 (20-30)	26.88 (23-30)
Habitual daily caffeine intake (mg)	113.06 (0-300)	105.13 (5-340)
Taking acetylcholinesterase inhibitors	0	0
Taking dopaminergic medication	24 (100%)	0

Table 5.1 Comparison of PD and healthy control participant (from chapter 4) demographics

All participants had been stable on their current medication for at least three months and there were no medication changes during the trial.

PD Recruitment

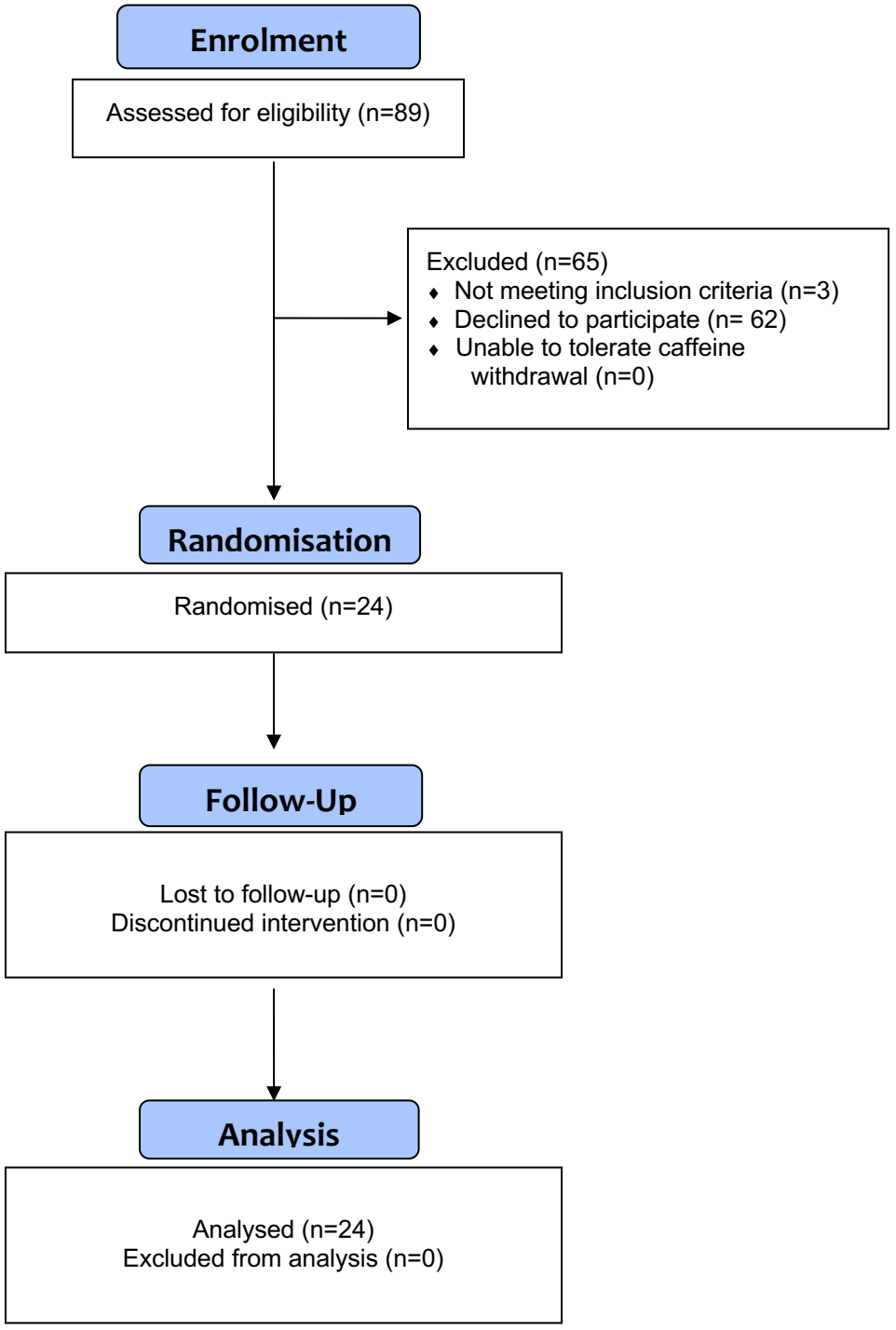

Figure 5.1 Recruitment phases for PD participants in this placebo controlled, cross over trial. Adapted from Consolidated Standards of Reporting Trials Group (Moher et al., 2001)

5.2.2 Procedure

A single blind, crossover trial compared 100mg caffeine (Proplus) tablets dissolved in instant decaffeinated coffee, with instant decaffeinated coffee. The coffee was served with or without artificial sweetener as per patient preference but consistently given across the trial. Milk was not offered. The drink was served at a temperature range of between 50 - 60ºC, which was confirmed by measurement with a thermometer. This ensured the drink was hot but not too hot for safe consumption.

Participants attended for baseline testing on day 1 without any dietary caffeine restriction. Following testing they were given a supply of either decaffeinated coffee and/or decaffeinated tea to cover the trial duration (as per their consumption preference) and requested to not ingest caffeine containing foods such as tea, coffee, chocolate etc. for the remainder of the trial (9 days) but could freely consume the decaffeinated tea/coffee we supplied them. On day seven (i.e. 1 week free from caffeine) participants repeated testing to assess for effects of caffeine withdrawal on attention and allow task familiarisation so that the effect of learning on subsequent performance was minimised. On day eight participants received either caffeinated or decaffeinated coffee and testing started 60 minutes later. In the interim, participants would wait in a quiet waiting room with books and magazines for interest if desired. On day nine the participants received the alternative type of coffee (caffeinated or decaffeinated whichever not already had) and began testing 60 minutes following consumption. Testing was performed within 15 minutes of the same time on all days.

5.2.3 Task

The task battery consisted of:

i. The Montreal Cognitive Assessment (MoCA)
ii. Digit span

iii. Simple reaction time
iv. Choice reaction time
v. The rapid serial visual presentation (RSVP) paradigm
vi. Stroop task
vii. Walking while talking test (WWT)

5.3 Results

5.3.1 Alerting attention

Parkinson' disease

A paired-samples t-test was used to determine whether there was a statistically significant mean difference between caffeine versus placebo on ***simple reaction time*** scores. Data are mean ± standard deviation, unless otherwise stated. The assumption of normality was not violated, as assessed by Shapiro-Wilk's test (p = 0.10). There was no statistically significant change between reaction time whilst on caffeine (339 ms ± 59) compared to placebo (348 ms ± 75), -9 ms, 95% CI [-28, 10], t(23) = -0.96, p = 0.35, d = 0.20.

A paired-samples t-test was used to determine whether there was a statistically significant mean difference between caffeine versus placebo on ***choice reaction time*** scores. Data are mean ± standard deviation, unless otherwise stated. The assumption of normality was not violated, as assessed by Shapiro-Wilk's test (p = 0.33). There was no statistically significant change between reaction time whilst on caffeine (558 ms ± 97) compared to placebo (563 ms ± 109), -5 ms, 95% CI [-24, 14], t(23) = -0.52, p = 0.35, d = 0.11.

A paired-samples t-test demonstrated no statistically significant mean change in error rate on ***choice reaction time*** when subjects ingested caffeine (1.8% ± 2.1) compared to placebo (2.0% ± 2.0), -2.2%, 95% CI [-0.66, 0.21], t(23) = -1.05, p = 0.30, d = 0.21.

A paired-samples t-test was used to determine whether there was a statistically significant mean difference between caffeine versus placebo on ***cognitive reaction time*** scores. Data are mean ± standard deviation, unless otherwise stated. The assumption of normality was not violated, as assessed by Shapiro-Wilk's test (p = 0.53). There was no statistically significant change between reaction time whilst on caffeine (219 ms ± 72) compared to placebo (215 ms ± 77), 4 ms, 95% CI [-14, 21], t(23) = -0.47, p = 0.64, d = 0.10.

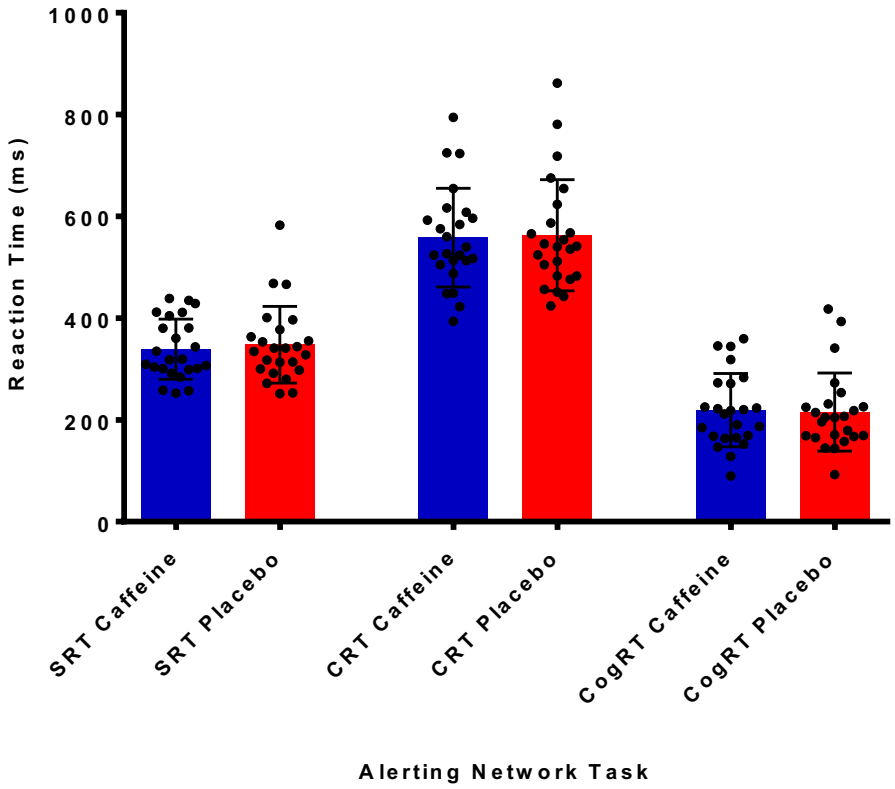

Figure 5.2a PD mean reaction time on simple reaction time (SRT), choice reaction time (CRT) and cognitive reaction time (CogRT). No significant difference was observed.

<u>PD versus aged matched healthy participants whilst on placebo</u>

A Welch t-test was run to determine if there were differences on ***simple reaction time*** scores between PD and aged matched healthy participants, due to the assumption of homogeneity of variances being violated, as assessed by Levene's test

for equality of variances (p = 0.05). There were 24 PD participants and 42 aged matched healthy participants. Data are mean ± standard deviation, unless otherwise stated. The simple reaction time scores were faster for healthy participants (303 ms ± 42) than PD participants (348 ms ± 75), a statistically significant difference, -45 ms, 95% CI [-74, -16], t(31.35) = -2.69, p = 0.01, d = 0.74

An independent samples t-test was run to determine if there were differences on ***choice reaction time*** scores between PD and aged matched healthy participants. There was homogeneity of variances, as assessed by Levene's test for equality of variances (p = 0.053). There were 24 PD participants and 42 aged matched healthy participants. Data are mean ± standard deviation, unless otherwise stated. There was no statistically significant change between healthy participants (524 ms ± 66) and PD participants (563 ms ± 109), -39 ms, 95% CI [-82, 5], t(64) = -1.76, p = 0.08, d = 0.43

An independent samples t-test was run to determine if there were differences on ***choice reaction time*** error rates between PD and aged matched healthy participants. There was homogeneity of variances, as assessed by Levene's test for equality of variances (p = 0.09). There were 24 PD participants and 42 aged matched healthy participants. Data are mean ± standard deviation, unless otherwise stated. There was no statistically significant change between healthy participants (1.2 ± 1.4) and PD participants (2.0 ± 2.0), -0.8, 95% CI [-1.7, 0.4], t(64) = -1.90, p = 0.06, d = 0.46

An independent samples t-test was run to determine if there were differences on ***cognitive reaction time*** scores between PD and aged matched healthy participants. There was homogeneity of variances, as assessed by Levene's test for equality of variances (p = 0.34). There were 24 PD participants and 42 aged matched healthy participants. Data are mean ± standard deviation, unless otherwise stated. There was no statistically significant change between healthy participants

(222 ms ± 55) and PD participants (215 ms ± 77), 6 ms, 95% CI [-26, 439], t(64) = 0.39, p = 0.70, d = 0.10

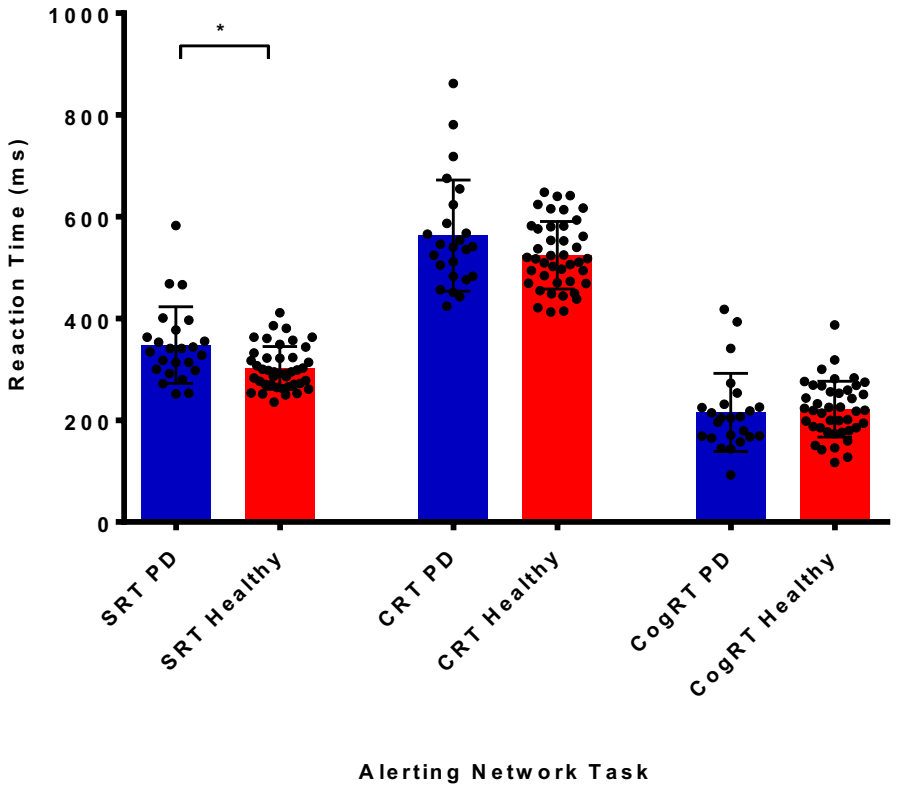

Figure 5.2b Comparing PD and healthy elderly mean reaction time on simple reaction time (SRT), choice reaction time (CRT) and cognitive reaction time (CogRT). There was a significantly faster simple reaction time for healthy participants compared to PD participants.

5.3.2 Orienting attention

Parkinson' disease

A three-way repeated measures ANOVA was run to determine the effect of caffeine on accuracy at different time points on the Rapid Serial Visual presentation task. Mauchly's test of sphericity indicated that the assumption of sphericity was met for the three-way interaction, $\chi 2(20) = 29.73$, p = 0.08. There was no statistically significant three-way interaction between caffeine, task and time, F(6, 258) = 1.11, p = 0.36.

There was no statistically significant two-way interaction between task and intervention $F(1, 23) = 3.49$, $p = 0.08$ or time and intervention $F(6, 138) = 1.86$, $p = 0.09$. As expected there was a significant interaction between task and time $F(2.97, 68.20) = 11.57$, $p < 0.01$.

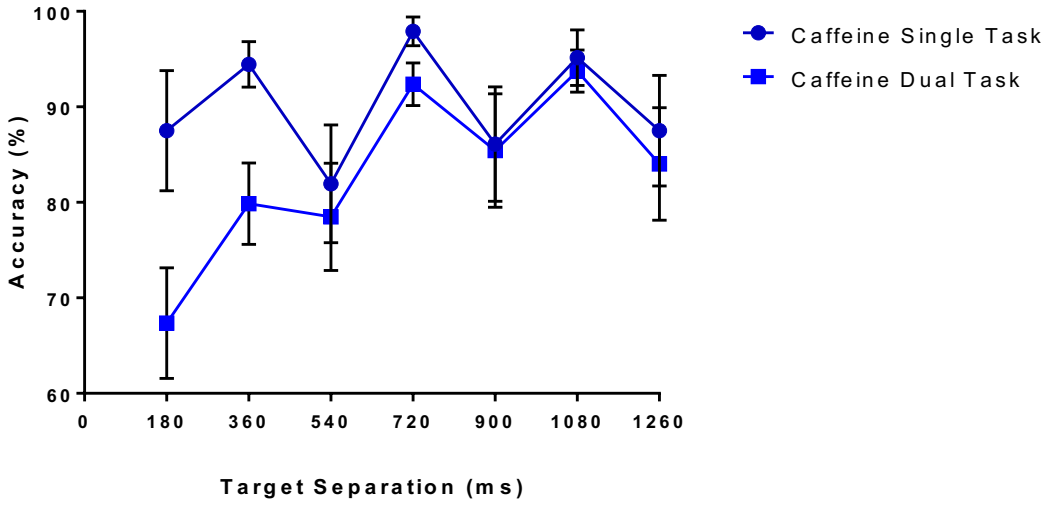

Figure 5.3a PD mean accuracy on single and dual tasks of the Rapid Serial Visual Presentation paradigm whilst on caffeine. Error bars represent standard error of the mean. The area of interest is the point of intersect between single and dual task result lines. This represents the "attentional blink", the time required to attend a primary target before disengaging and attending to a second target accurately. Under caffeine the attentional blink is 900 ms.

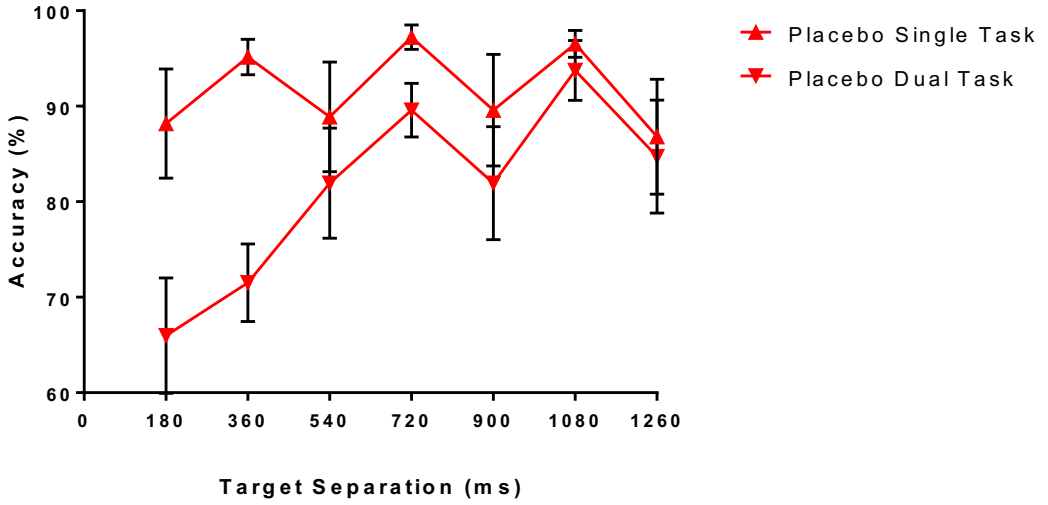

Figure 5.3b PD mean accuracy on single and dual tasks of the Rapid Serial Visual Presentation paradigm whilst on placebo. Error bars represent standard error of the mean. Under placebo the attentional blink is 1080 ms.

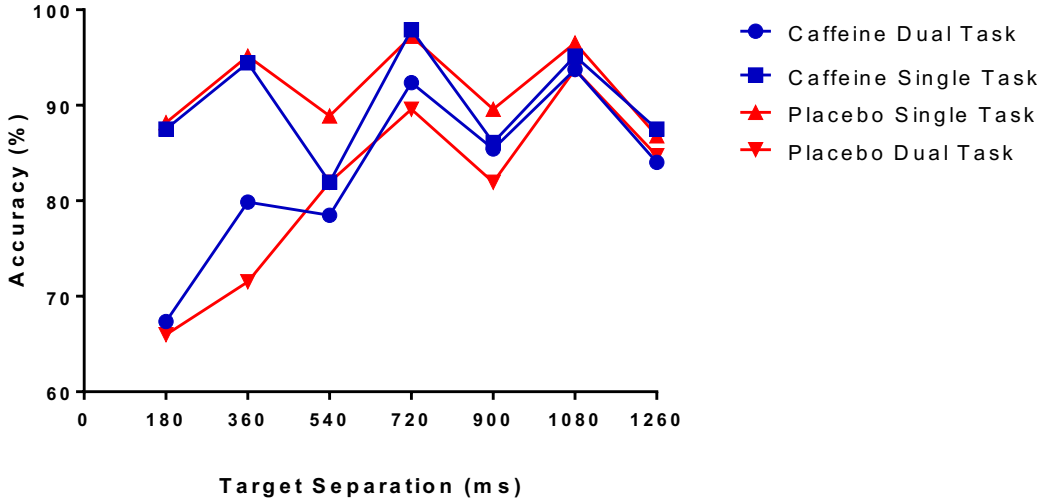

Figure 5.3c PD mean accuracy on single and dual tasks of the Rapid Serial Visual Presentation paradigm comparing caffeine with placebo.

PD versus aged matched healthy participants whilst on placebo

A three-way ANOVA was run to determine the difference in accuracy between PD and aged matched healthy participants, at different time points on the Rapid Serial Visual presentation task. There was no statistically significant three-way interaction between participant group, task and time, $F(6, 896) = 0.59$, $p = 0.74$.

As expected there was a statistically significant simple two-way interaction between task and time $F(6, 896) = 5.84$, $p < 0.01$. There was no statistically significant two-way interactions between participant group and task $F(1, 896) < 0.01$, $p = 0.99$ or participant group and time $F(6, 896) = 1.36$, $p = 0.23$.

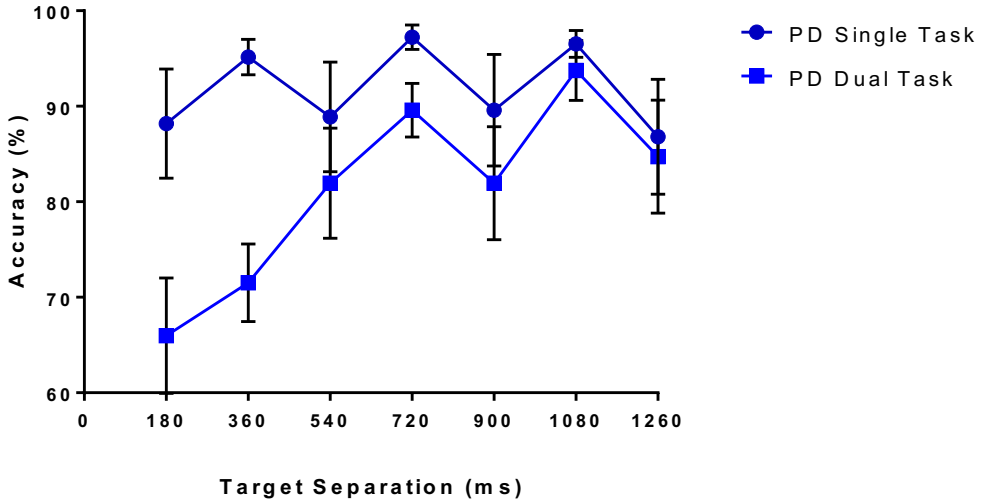

Figure 5.3d PD placebo Mean accuracy on single and dual tasks of the Rapid Serial Visual Presentation paradigm. Error bars represent standard error of the mean. The area of interest is the point of intersect between single and dual task result lines. This represents the "attentional blink", the time required to attend a primary target before disengaging and attending to a second target accurately. Under caffeine the attentional blink is 1080 ms.

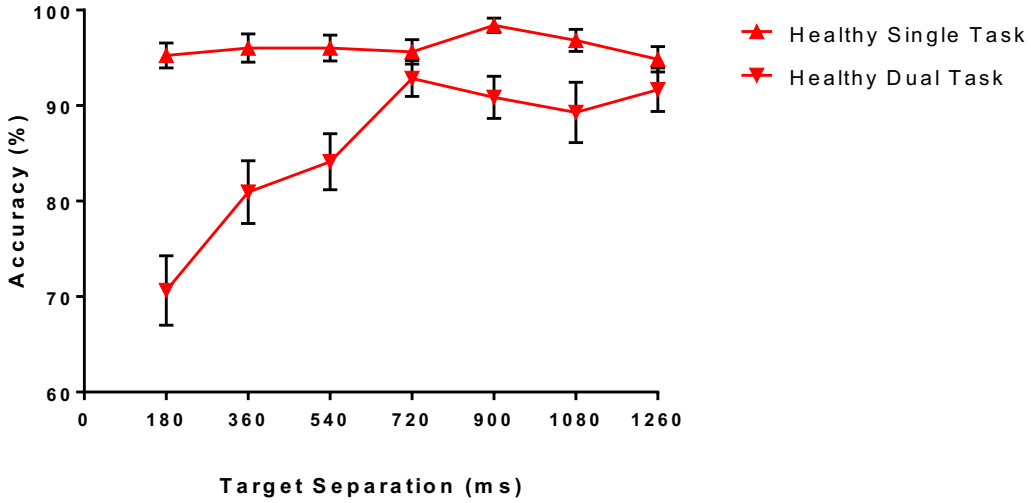

Figure 5.3e Healthy elderly placebo mean accuracy on single and dual tasks of the Rapid Serial Visual Presentation paradigm. Error bars represent standard error of the mean. The attentional blink is 720 ms although it appears to worsen as target separation increases until 1260 ms.

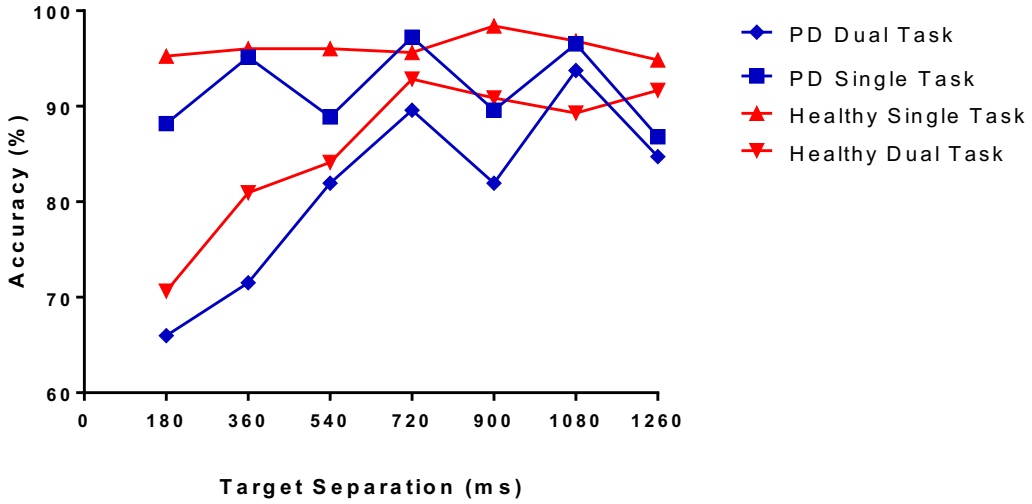

Figure 5.3f Comparing PD and healthy elderly mean accuracy on single and dual tasks of the Rapid Serial Visual Presentation paradigm. There is no statistical difference in the attention blink.

5.3.3 Executive attention

Parkinson' disease

A paired-samples t-test was used to determine whether there was a statistically significant mean difference between caffeine versus placebo on ***Stroop reaction time*** scores. Data are mean ± standard deviation, unless otherwise stated.

The assumption of normality was not violated, as assessed by Shapiro-Wilk's test for neutral (p = 0.19), incongruent (p = 0.06) and total (p = 0.44) conditions. There was no statistically significant change in reaction time during the neutral condition whilst on caffeine (974 ms ± 263) compared to placebo (1021 ms ± 267), -47 ms, 95% CI [-94, 26], t(23) = -2.03, p = 0.06, d = 0.41.

There was no statistically significant change in reaction time during the incongruent condition whilst on caffeine (1182 ms ± 403) compared to placebo (1230 ms ± 381), -47 ms, 95% CI [-116, 21], t(23) = -1.44, p = 0.16, d = 0.29.

There was no statistically significant change in average Stroop reaction time whilst on caffeine (1078 ms ± 327) compared to placebo (1125 ms ± 317), -47 ms, 95% CI [-101, 7], t(23) = -1.79, p = 0.09, d = 0.37.

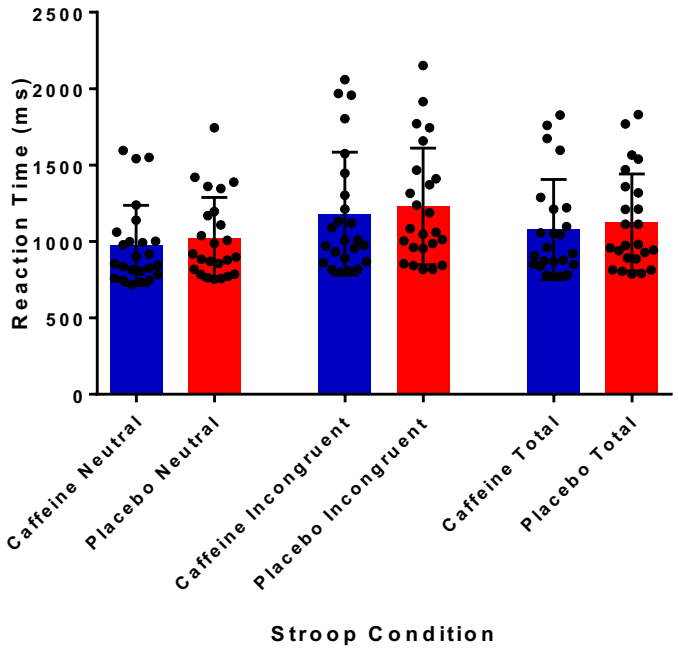

Figure 5.4a PD reaction time performance on the Stroop task. No significant difference was observed.

PD versus aged matched healthy participants whilst on placebo

A Welch t-test was run to determine if there were differences on ***Stroop reaction time*** scores between PD and aged matched healthy participants, due to the assumption of homogeneity of variances being violated, as assessed by Levene's test for equality of variances under neutral ($p < 0.01$), incongruent ($p < 0.01$) and total ($p < 0.01$) conditions. There were 24 PD participants and 42 aged matched healthy participants. Data are mean ± standard deviation, unless otherwise stated. The neutral condition scores were faster for healthy participants (853 ms ± 129) than PD participants (1021 ms ± 267), a statistically significant difference, -168 ms, 95% CI [-286, -49], $t(29.23) = -2.90$, $p < 0.01$, $d = 1.73$

The incongruent condition scores were faster for healthy participants (982 ms ± 174) than PD participants (1230 ms ± 381), a statistically significant difference, -248 ms, 95% CI [-416, -79], $t(28.57) = -3.01$, $p < 0.01$, $d = 0.84$

The total condition scores were faster for healthy participants (917 ms ± 149) than PD participants (1125 ms ± 317), a statistically significant difference, -208 ms, 95% CI [-348, -67], t(28.89) = -3.03, p < 0.01, d = 0.84

Due to the use of multiple t-test statistics a Bonferroni correction (Armstrong, 2014) produces an adjusted alpha level of 0.0167 (0.05/3) which means all the tests described above remain statistically significant.

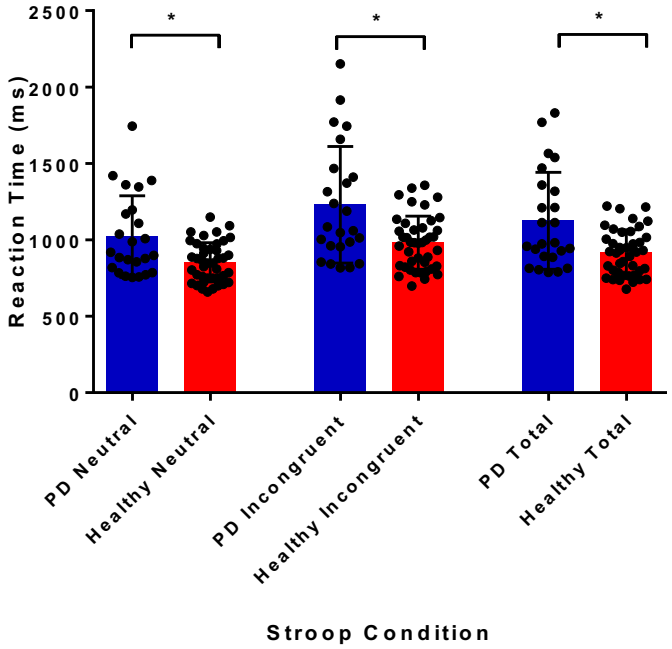

Figure 5.4b Comparing PD and healthy elderly reaction time performance on the Stroop task. There was a significant difference between PD and healthy participant reaction times in each Stroop condition.

5.3.4 Walking while talking

Parkinson' disease

A related samples Sign test was used to determine whether there was a statistically significant median difference between caffeine versus placebo on *walking while talking* times. Data are medians unless otherwise stated.

There was no statistically significant change in walking times on caffeine (16.6 s) compared to placebo (17.2) p = 0.54. There was no statistically significant change in walking while talking times on caffeine (23.9 s) compared to placebo (23.6) p = 0.15.

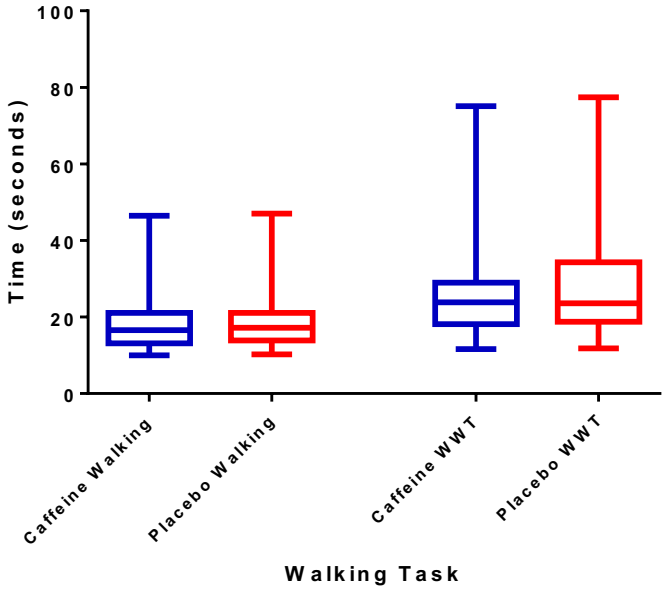

Figure 5.5a PD walking while talking task performance. No significant difference was observed.

PD versus aged matched healthy participants whilst on placebo

An independent samples t-test was run to determine if there were differences on ***walking*** time scores between PD and aged matched healthy participants. There was homogeneity of variances, as assessed by Levene's test for equality of variances ($p = 0.34$). There were 24 PD participants and 42 aged matched healthy participants. Data are mean ± standard deviation, unless otherwise stated. There was no statistically significant change between healthy participants (16.0 s ± 6.0) and PD participants (18.7 s ± 8.2), -2.7 s, 95% CI [-6.2, 0.8], $t(64) = -1.55$, $p = 0.13$, $d = 0.38$

A Welch t-test was run to determine if there were differences on ***walking while talking*** time scores between PD and aged matched healthy participants, due to the assumption of homogeneity of variances being violated, as assessed by Levene's test for equality of variances ($p < 0.01$). There were 24 PD participants and 42 aged matched healthy participants. Data are mean ± standard deviation, unless otherwise stated. The walking while talking time scores were faster for healthy

participants (16.1 s ± 6.1) than PD participants (28.7 s ± 15.4), a statistically significant difference, -12.6 s, 95% CI [-19.3, -5.9], t(27.19) = -3.85, p < 0.01, d = 1.08

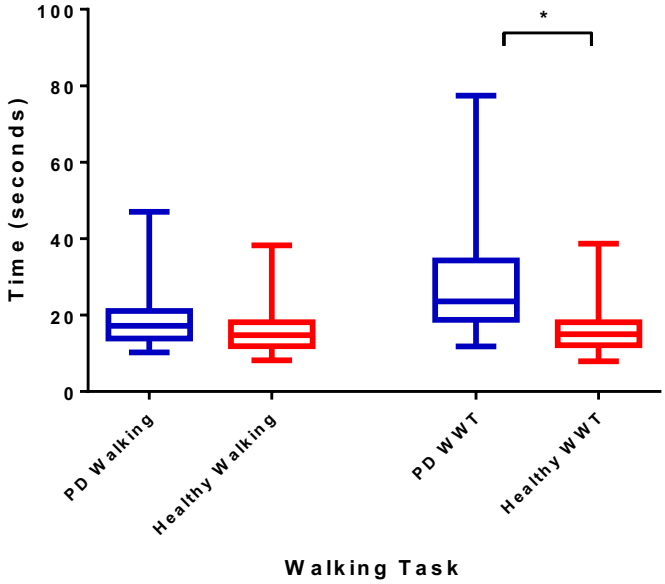

Figure 5.5b Comparing PD and healthy elderly walking while talking task performance. Almost identical performance on caffeine and placebo for aged matched healthy controls but PD was significantly slower on walking while talking despite a relatively good walking time.

5.3.5 Digit Span

Parkinson' disease

A related samples Wilcoxon signed rank test was used to determine whether there was a statistically significant median difference between caffeine versus placebo on *Digit span* scores. Data are medians unless otherwise stated.

There was no statistically significant change on forward digit span length on caffeine (12.5 digits) compared to placebo (12.0 digits) z = 0.22, p = 0.83.

There was no statistically significant change on backward digit span length on caffeine (7.0 digits) compared to placebo (7.5 digits) z = 1.24, p = 0.21.

There was no statistically significant change on total digit span length on caffeine (18.5 digits) compared to placebo (19.0 digits) z = 0.80, p = 0.42.

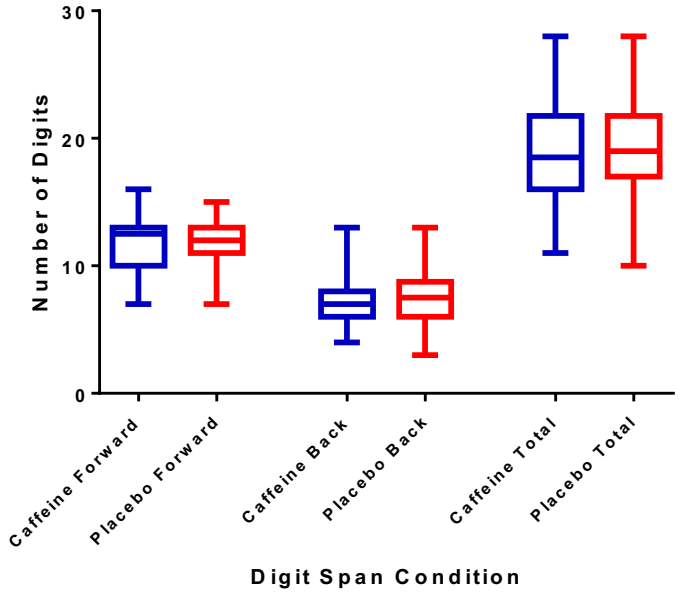

Figure 5.6a PD digit span performance. Error bars represent standard error of the mean.

PD versus aged matched healthy participants whilst on placebo

An independent samples t-test was run to determine if there were differences on ***digit span*** scores between PD and aged matched healthy participants. There was homogeneity of variances, as assessed by Levene's test for equality of variances for forward ($p = 0.33$), backward ($p = 0.85$) and total ($p = 0.96$) conditions. There were 24 PD participants and 42 aged matched healthy participants. Data are mean ± standard deviation, unless otherwise stated. There was no statistically significant change on forward digit span between healthy participants (10.9 ± 2.2) and PD participants (11.8 ± 2.0), -0.82, 95% CI [-1.9, 0.3], $t(64) = -1.49$, $p = 0.14$, $d = 0.39$

There was no statistically significant change on backward digit span between healthy participants (7.7 ± 2.2) and PD participants (7.6 ± 2.5), 0.85, 95% CI [-1.0, 1.3], $t(64) = 0.19$, $p = 0.85$, $d = 0.05$

There was no statistically significant change on total digit span between healthy participants (18.7 ± 3.8) and PD participants (19.3 ± 4.0), 0.78, 95% CI [-2.7, 1.3], t(64) = -0.71, p = 0.48, d = 0.18

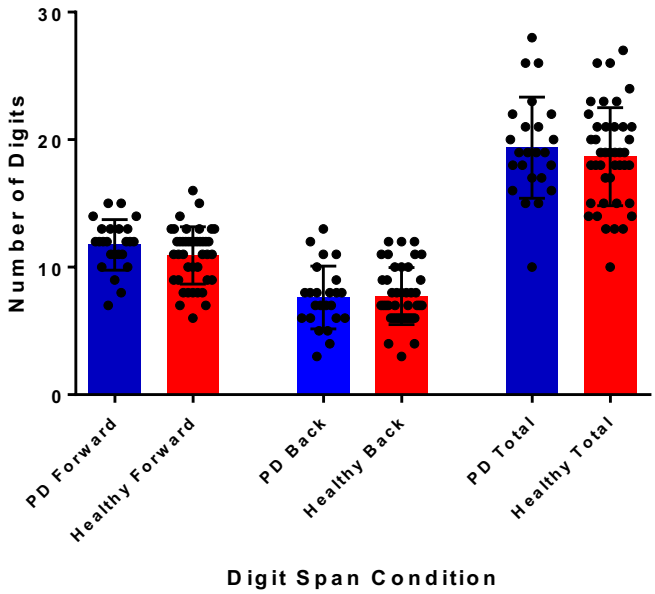

Figure 5.6b Comparing PD and healthy elderly digit span performance. Error bars represent standard error of the mean.

5.3.6 Correlations

Parkinson' disease

A Spearman's rank-order correlation was run to assess the relationship between *cognitive reaction time* and habitual caffeine consumption. Preliminary analysis showed the relationship to be monotonic, as assessed by visual inspection of a scatterplot. There was a positive correlation between habitual caffeine consumption and cognitive reaction time, $r_s(24) = 0.57$, $p < 0.01$. As habitual caffeine intake increased, cognitive reactions times increased i.e. were slower.

There was no statistically significant difference in age between the PD and healthy control participants. There was no effect of intervention crossover order as a between subjects variable for any of the tests.

5.3.7 Post hoc power analyses

Parkinson' disease

To assess whether my non-significant results were the result of type II error, I conducted post hoc power analyses using G*Power (Faul et al., 2007) with power (1 - β) set at 0.80 and α = 0.05, two-tailed.

To test my hypothesis with statistical significance at the 0.05 level:

- alerting attention sample sizes would be N = 198, 651 and 180 for SRT, CRT and CogRT, respectively
- orienting attention sample sizes would be N = 26
- executive attention sample sizes would be N = 49, 95 and 59 for congruent, incongruent and average Stroop, respectively
- digit span sample sizes would be N = 2471, 58 and 101 for forward, backward and total digit span, respectively
- WWT sample sizes would be N = 147 and 53 for walking and WWT, respectively

5.4 Discussion

5.4.1 The effect of caffeine on attention in PD

This study investigated the effect of caffeine on each individual subtype of attention, in PD participants. In direct contradiction to my original hypotheses, caffeine did not significantly improve performance on tasks testing any attentional network, real work tasks of attention or functional tasks of working memory. Below I explore the reasons for this.

Tasks of alerting attention, the simple, choice and cognitive reaction time, demonstrated no objective improvements following caffeine ingestion. There are

only two other published PD caffeine trials (Postuma et al., 2017, Postuma et al., 2012) both published by the same group. Using 200 – 400mg caffeine a day over 6 weeks showed no improvement on subjective measures of attention whilst over 6 months there was a modest *subjective* improvement as assessed by questionnaire. Our studies differ in that I used a much lower dose of caffeine, 100mg and fully withdrew participants whereas Postuma allowed habitual caffeine use to continue. Not withdrawing participants from caffeine in the control group is a critical failing in their study design as rather than comparing the effect of caffeine against control i.e. caffeine free participants, in actuality they compared habitual dose caffeine against high dose caffeine (habitual caffeine intake plus 200-400mg/day).

A physiological explanation as to why alerting attention did not improve may simply lie in the anatomical co-localisation of dopaminergic D2 receptors with adenosine A2A receptors. If dopaminergic neurons are/or receptors are down regulated then it stands to reason that co-localised adenosine receptors would be down regulated too, and this has been demonstrated in the dorsal striatum (Svenningsson et al., 1999).

Executive attention as measured by the Stroop task demonstrated no improvement following caffeine ingestion. Caffeine has been proposed to mediate its alerting effects through dopamine up regulation (Brunye et al., 2010), which may explain this negative result. In PD, at diagnosis, dopamine production is approximately 70% of aged matched controls and this diminishes with time (Fearnley and Lees, 1991). If there are already insufficient dopaminergic neurons, working at maximal capacity, up regulation will not be possible, hence the lack of effect. Ideally this theory would have been supported by a positive correlation between Stroop task performance and the Unified Parkinson's Disease rating scale, a marker of dopaminergic function but unfortunately I did not have the foresight to record this data.

The only significant correlation was a slower Cognitive Reaction Time score with increased habitual caffeine intake. It is difficult to ascribe significance to this. As caffeine consumers are thought to self titrate their intake to obtain a therapeutic effect, high users will likely require a larger dose than low habitual users to generate the same effect. Another variable is size, I gave everyone the same dose of caffeine whereas other studies protocol caffeine dose according to body mass. This correlation could represent a difference in effect due to variation in therapeutic caffeine level.

As expected there was no significant effect or trend towards caffeine having an effect on orienting attention or digit span. There was no effect or trend on the walking while talking task, a real world task of attention. This has been validated as a surrogate marker of falls in PD, a significant source of distress and morbidity (LaPointe et al., 2010, Verghese et al., 2002a). Given the lack of attentional enhancement demonstrated in the computerised tasks, it is unsurprising there was no improvement on walking while talking.

5.4.2 PD versus aged matched healthy participants

Comparison of the PD group with healthy aged matched controls (from Chapter 4) elicited one surprise. As predicted executive and to a lesser degree orienting attention appeared impaired in the PD cohort, as did walking while talking time. Digit span was similar, which is not unexpected given the MoCA scores were similar. However, surprisingly, cognitive reaction times were approximately the same.

An early and prominent feature of PD cognitive decline is reduced processing speed and one would expect this to manifest as a prolonged cognitive reaction time, especially as the orienting and executive reaction times are impaired. Examining the individual components of cognitive reaction time, it is evident both simple and choice reaction time are slower in PD than healthy controls but by the same

proportion, indicating the difference is due to motor slowness affecting the response time rather than an impairment in alerting attention or processing speed.

Previous research in this area has been limited in its interpretation by the lack of a *cognitive* reaction time calculation (Jordan et al., 1992, Jahanshahi et al., 1992b, Jahanshahi et al., 1992a), which leaves assessment of simple and choice reaction time confounded by impaired motor function speed, the essential feature of PD. This is a novel finding, which I have not found described elsewhere in the literature; PD sufferers have impaired orienting and executive attention but normal alerting attention. If alerting attention were not impaired then one would not expect caffeine to improve this attentional domain, as it might already be functioning optimally.

Interestingly performance of PD participants in a real world task of attention, the walking while talking task, demonstrated no difference in walking time but a significant difference in walking while talking time, a surrogate marker of falls (LaPointe et al., 2010). The disparity between the 2 test indices suggest motor speed is not a factor of the significant result, instead it is the consequence of impaired attention in PD. Having systematically assessed each attentional domain, it can be deduced impaired walking while talking time corresponds best with impaired executive attention. Recent phase 2 clinical trials have demonstrated promise for Rivastigmine as a remedy for gait instability. It acts as a cholinesterase inhibitor, increasing cholinergic transmission which enhances orienting attention (Henderson et al., 2016). My data confers cholinesterase inhibitors may be of some benefit at improving gait stability but optimising dopaminergic pathways will be of the greatest benefit, however, this may not be possible in this population.

5.4.3 Limitations

The PD and healthy controls were evenly matched for aged, MoCA score and habitual caffeine intake, however, the PD cohort contained a male predominance whilst the control group contained a female predominance. It is possible this difference in demographics contributed to the differences in attention between the two groups although sex has not been reported as a predictor of attentional performance in the literature.

Post hoc power analysis indicates the study is somewhat underpowered and would require the sample size to be at least tripled to be confident accepting the null hypothesis is valid and caffeine has no beneficial effect on attention in PD.

The collection of further data, specifically the UPDRS score and the incidence of withdrawal symptoms on caffeine withdrawal in PD participants, would have allowed for further pertinent correlations to be undertaken. It is only with hindsight that I can see their relevance and this can be used to guide future data collection.

5.4.4 Conclusion

For decades, based on epidemiological data, caffeine has been lauded as a modifiable, dietary supplement protective against PD. As greater consideration is given to this topic, to date the association has not been validated by clinical trials evaluating the effect of chronic caffeine ingestion on PD. My study has clearly demonstrated acute caffeine produces no improvement on attention in PD.

Whilst a negative result is disappointing, as the implications of a positive result would be the potential to improve the lives of people with PD, it is also thought provoking. It suggests, possibly, caffeine mediates its effect through dopamine, whether directly or indirectly, which help explain not only its alerting affects but also pharmacodynamics properties of tolerance, withdrawal and reward. To test this hypothesis future studies could repeat the experimental paradigm in

populations with impaired attention but intact dopaminergic pathways such as multiple sclerosis sufferers.

Chapter 6

The utility of caffeine as an attentional enhancer in multiple sclerosis: a pilot study

6.1 Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system, characterised by demyelination with partial axonal preservation (Lassmann et al., 2007). This autoimmune disease is mediated by T-lymphocytes directed against myelin sheath antigens, which triggers a cascade of focal demyelination, oligodendrocyte destruction and neuro-degeneration (Bjartmar et al., 2000, Hohlfeld and Wekerle, 2004). Current treatments for MS are aimed at disease modification and therefore target the T-cell inflammatory response. Coffee and by extension caffeine have been the subject of case controlled studies evaluating whether they yield a protective effect in MS although there is no consensus amongst the data (Massa et al., 2013, Hedstrom et al., 2016) and no physiological reason why caffeine would produce a disease modifying effect. However, the use of caffeine as a *symptomatic treatment* for MS cognitive problems is worthy of exploration as there is a physiological mechanism to support its effect, explored below, and it has the potential to improve quality of life.

In the United Kingdom MS is the most common cause of disability in people aged less than 40 years old. Along with progressive neurological disability, it is being increasingly recognised that cognitive and psychological dysfunction is a prominent feature of the disease. Fatigue is a common and debilitating symptom of MS, affecting between 65 and 92% (Branas et al., 2000), independent of physical disability (Alvarenga-Filho et al., 2015). It has a high impact on the quality of life, often described as the most debilitating symptom (Krupp et al., 1988, Fisk et al.,

1994), affecting productivity and employment (Flensner et al., 2008) as well as negative effects on social and physical function (Fisk et al., 1994).

Fatigue is an ambiguous term, which can be defined as "subjective lack of physical and/or mental energy that is perceived by the individual to interfere with usual and desired activities" (Andreasen et al., 2010). Whilst a universally recognised classification remains elusive, it can broadly be categorised into mental (often referred to as central) (Silverman et al., 2010) and physical (often referred to as peripheral) (Krupp and Pollina, 1996). Physical fatigue represents neuromuscular dysfunction and is not discussed further as this would not be amenable to attentional enhancement.

Mental fatigue can be further classified into primary and secondary. Primary is related to the pathological disease process, in this case, demyelination of central nervous system axons. Secondary is related to MS associated complications such as depression, pain, sleep and medication side effects; there is a functional disturbance presumably mediated by a neurotransmitter imbalance (Chaudhuri and Behan, 2000). Clearly primary and secondary mental fatigue co-exist and it would be impossible to delineate the significance of one from the other. The lack of consensus on the medical definition of fatigue hinders objective measurement and treatment.

The pathophysiology of mental fatigue in MS has not been elucidated but it is increasingly postulated that disruption of the normal hypothalamic-pituitary axis and neurotransmitter pathways are the underlying basis of symptoms (Bol et al., 2010, Lucchinetti et al., 2011, Filippi et al., 2002). There are some studies, which correlate fatigue with a higher demyelinating lesion load on MRI although this is not an unequivocal association (Vercellino et al., 2009). Melatonin and hypocretin secretion, neuro-hormones both involved in wakefulness and arousal, can become deregulated in MS, with a difference in levels between cohorts with and without fatigue (Papuć et al., 2010, Melamud et al., 2012).

Disruption and/or dysfunction of the thalamo-striato-cortical network is proposed as the anatomical pathway responsible for fatigue resulting in neurotransmitter disruption between the striatum and prefrontal cortex (Alexander and Crutcher, 1990). Dysfunction of the thalamo-striato-cortical pathways correlates with impairment in the alerting and executive attentional networks as discussed in chapter 1. I postulate that fatigue, is in part a subjective manifestation of decreased attention and may be amenable to attentional enhancement.

Prokarin, a histamine and caffeine containing medication, has shown a modest improvement in MS fatigue scores compared to placebo (Gillson et al., 2002). The rationale behind the trial was the use of histamine analogues to exert increased mental alertness to improve fatigue. Unfortunately they did not adequately factor in the effect of caffeine or caffeine withdrawal into the analysis (Gillson et al., 2002). Modafinil a wakefulness promoting drug has been trialled for fatigue in MS with conflicting results (Littleton et al., 2010, Stankoff et al., 2005, Rammohan et al., 2002). This discrepancy is in part due to differences and inadequacies in the definition of fatigue. This results in heterogeneity of participant selection and fatigue measurement rendering the trials incomparable to each other. The difficulty in using questionnaires to assess fatigue such as the Modified Fatigue Impact Scale (Schiehser et al., 2015), is their score does not reflect fatigue in isolation but will also score for depression and cognitive impairment which may not respond to the intervention. As a result it can be unclear which of these three domains the intervention is improving.

Dopaminergic networks have been shown to be involved in fatigue, which has been proposed to occur due to loss of integrity of basal ganglia non-motor function, termed the dopamine imbalance theory (Dobryakova et al., 2015). This is interesting as non-motor functions of the basal ganglia contribute to attention, which gives weight to central fatigue being a manifestation of impaired attention. Amantadine, a weak dopamine agonist, is the only drug recommended for fatigue in MS by the National Institute of Clinical Excellence (NICE, 2014). From trial data its

effect was small (Group, 1987). The mechanism by which it improves fatigue is not clear but its dopaminergic activity may restore dopamine imbalance. Given caffeine indirectly increases dopaminergic activity through adenosine antagonism, it has the potential to be a potent enhancer of cognitive fatigue.

Cognitive impairment is a common finding in MS affecting between 40% to 65% (Bobholz and Rao, 2003). Whilst it can occur at any stage of the disease, it tends to be most pronounced during the secondary progressive phase. There is no specific pattern of cognitive impairment as demyelination can affect any part of the brain but typical domains affected are processing speed, attention, episodic memory and executive function (Chiaravalloti and DeLuca, 2008, Amato et al., 2006, Reuter et al., 2010, Feinstein et al., 1992, Kujala et al., 1997). Cognitive processing speed impairment emerges as the dominant feature and one can deduce how this would potentiate a negative effect on testing attention, episodic memory and executive function.

The pathophysiology of fatigue and impaired cognitive processing speed have been proposed to be, in part, related to loss of normal melatonin homeostasis and neurotransmitter dysregulation between the striatum and prefrontal cortex. Caffeine, given its properties as an adenosine antagonist, which indirectly antagonises the effects of melatonin, would be an obvious antidote, however, controlled clinical trials assessing its role in fatigue and cognitive impairment are lacking.

6.1.1 Aims

Due to the time constraints of a finite 3 year research fellowship, the main aim of the study was to pilot the feasibility of conducting a definitive trial in terms of recruitment and tolerance of caffeine withdrawal procedures within a MS population. This pilot study will also establish the suitability of the assessments for measuring attentional outcomes in this patient group.

As a secondary aim, the study will produce descriptive and comparative statistics to assess whether 100mg of caffeine compared to placebo improves attention in a pilot group of fully withdrawn MS participants with cognitive impairment or cognitive fatigue on computerised neuropsychology paradigms and functional tasks of attention.

6.1.2 Hypothesis

Acute caffeine ingestion will improve attention in the alerting and executive domain in people with MS.

6.2 Methods

6.2.1 Participants

Twelve MS participants were tested. They were recruited from a clinical research database held in North Bristol NHS Trust's MS service.

The inclusion criteria for MS patients were:

- an established diagnosis of MS
- subjective or objective cognitive impairment, including mental fatigue
- adequate vision to perform the tasks
- an adequate level of communication in written and verbal English
- independently mobile

The exclusion criteria for MS patients were:

- any concomitant serious illness likely to interfere with cognitive or physical performance
- inability to consent to research, in keeping with the Mental Capacity Act 2005
- loss of capacity to consent to research during the trial

Participants	12
Age	55 (38-70)
Sex	5 male : 7 female
Baseline MoCA	26.33 (24-30)
Habitual daily caffeine intake (mg)	73.92 (2-165)
Taking acetylcholinesterase inhibitors	0
Taking dopaminergic medication	0

Table 6.1 MS participant demographics

All participants had been stable on their current medication for at least three months and there were no medication changes during the trial.

MS Recruitment

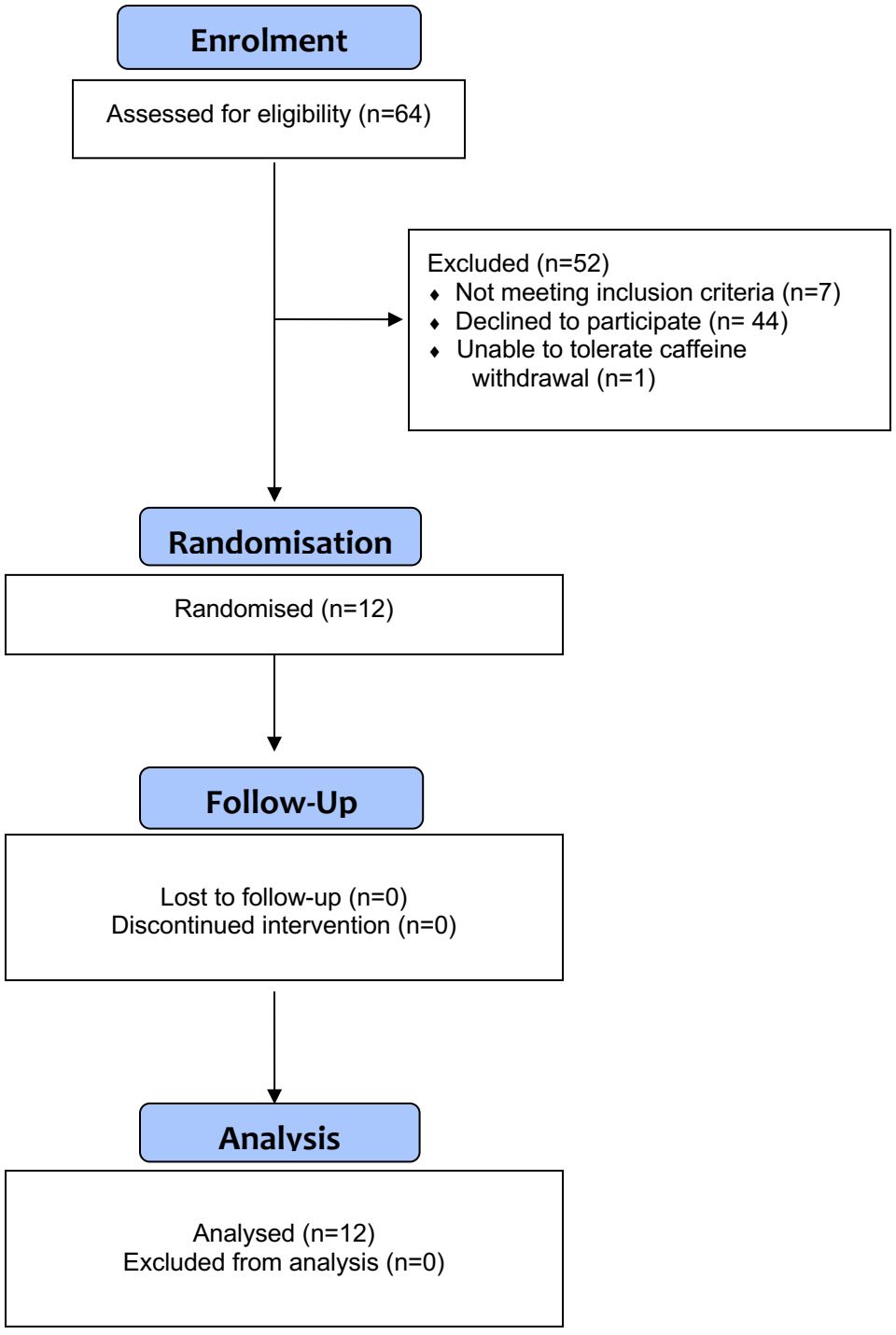

Figure 6.1 Recruitment phases for MS participants in this placebo controlled, cross over trial. Adapted from Consolidated Standards of Reporting Trials Group (Moher et al., 2001)

6.2.2 Procedure

A single blind, crossover trial compared 100mg caffeine (Proplus) tablets dissolved in instant decaffeinated coffee, with instant decaffeinated coffee. The coffee was served with or without artificial sweetener as per patient preference but consistently given across the trial. Milk was not offered. The drink was served at a temperature range of between 50 - 60ºC which was confirmed by measurement with a thermometer. This ensured the drink was hot but not too hot for safe consumption.

Participants attended for baseline testing on day 1 without any dietary caffeine restriction. Following testing they were given a supply of either decaffeinated coffee and/or decaffeinated tea to cover the trial duration (as per their consumption preference) and requested to not ingest caffeine containing foods such as tea, coffee, chocolate etc. for the remainder of the trial (9 days) but could freely consume the decaffeinated tea/coffee we supplied them. On day seven (i.e. 1 week free from caffeine) participants repeated testing to assess for effects of caffeine withdrawal on attention and allow task familiarisation so that the effect of learning on subsequent performance was minimised. On day eight participants received either caffeinated or decaffeinated coffee and testing started 60 minutes later. In the interim, participants would wait in a quiet waiting room with books and magazines for interest if desired. On day nine the participants received the alternative type of coffee (caffeinated or decaffeinated whichever not already had) and began testing 60 minutes following consumption. Testing was performed within 15 minutes of the same time on all days.

6.2.3 Task

The task battery consisted of:

i. The Montreal Cognitive Assessment (MoCA)

ii. Digit span
iii. Simple reaction time
iv. Choice reaction time
v. The rapid serial visual presentation (RSVP) paradigm
vi. Stroop task
vii. Walking while talking test (WWT)

Data analysis – data analysis was conducted using the tests described below. As this was a feasibility study, the primary objective was to provide data to estimate the sample size required to design a definitive randomised controlled trial with adequate power. Secondary objectives included collecting and synthesising data from which the pattern of effects could be evaluated, as this could consequentially affect trial design. Therefore we have carried out inferential statistics and also plotted individual data points where appropriate.

6.3 Results

6.3.1 Alerting attention

A paired-samples t-test was used to determine whether there was a statistically significant mean difference between caffeine versus placebo on ***simple reaction time*** scores. Data are mean ± standard deviation, unless otherwise stated.
The assumption of normality was not violated, as assessed by Shapiro-Wilk's test ($p = 0.41$). There was no statistically significant change between reaction time whilst on caffeine (322 ms ± 40) compared to placebo (326 ms ± 46), -4 ms, 95% CI [-18, 10], $t(11) = -0.68$, $p = 0.52$, $d = -0.20$.

A paired-samples t-test was used to determine whether there was a statistically significant mean difference between caffeine versus placebo on ***choice reaction time*** scores. Data are mean ± standard deviation, unless otherwise stated.

The assumption of normality was not violated, as assessed by Shapiro-Wilk's test (p = 0.85). There was no statistically significant change between reaction time whilst on caffeine (544 ms ± 102) compared to placebo (567 ms ± 111), -423 ms, 95% CI [-47, 0], t(11) = -2.18, p = 0.054, d = -0.66.

A paired-samples t-test demonstrated no statistically significant mean change in error rate on ***choice reaction time*** when subjects ingested caffeine (1.6% ± 1.3) compared to placebo (2.4% ± 2.4), -0.8%, 95% CI [-2.3, 0.6], t(11) = -1.28, p = 0.23, d = -0.39. There was no correlation with age, sex, MoCA score or habitual caffeine consumption.

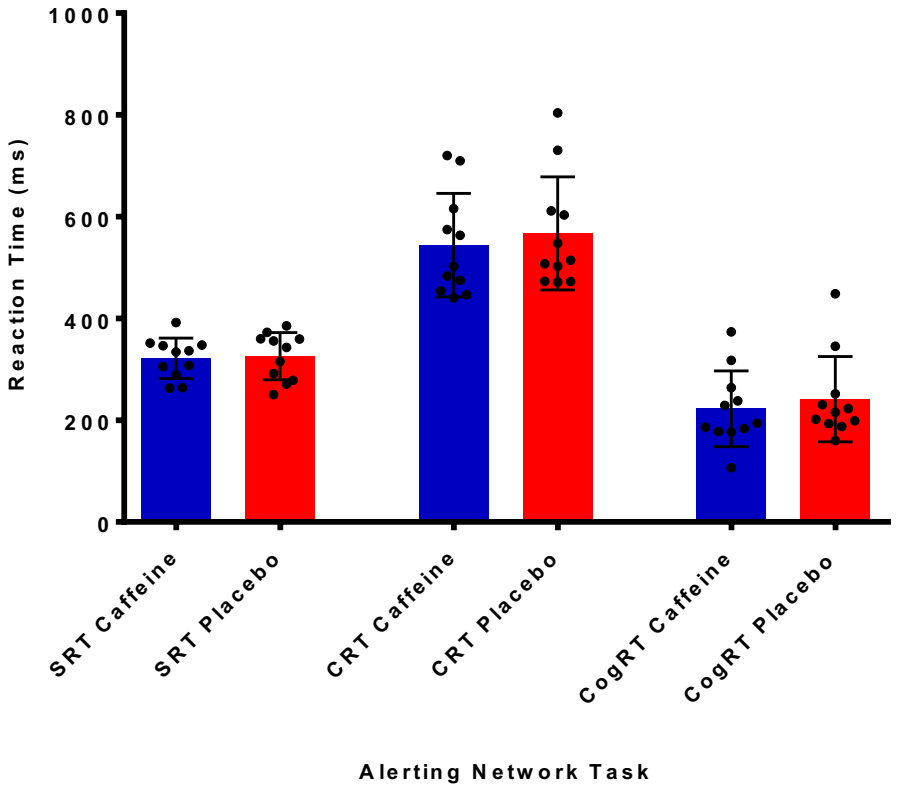

Figure 6.2 MS mean reaction time on simple reaction time (SRT), choice reaction time (CRT) and cognitive reaction time (CogRT). Reactions times were non-significantly improved by caffeine.

A paired-samples t-test was used to determine whether there was a statistically significant mean difference between caffeine versus placebo on ***cognitive reaction***

time scores. Data are mean ± standard deviation, unless otherwise stated. The assumption of normality was not violated, as assessed by Shapiro-Wilk's test (p = 0.73). There was no statistically significant change between reaction time whilst on caffeine (223 ms ± 74) compared to placebo (241 ms ± 84), -18.911 ms, 95% CI [-42, 4], t(11) = -1.81, p = 0.10, d = -0.55.

6.3.2 Orienting attention

A three-way repeated measures ANOVA was run to determine the effect of caffeine on accuracy at different time points on the ***Rapid Serial Visual presentation task***. Mauchly's test of sphericity indicated the assumption of sphericity was met for the three-way interaction, $\chi 2(20) = 19.41$, p = 0.53. There was no statistically significant three-way interaction between caffeine, task and time, F(6, 66) = 0.45, p = 0.84.

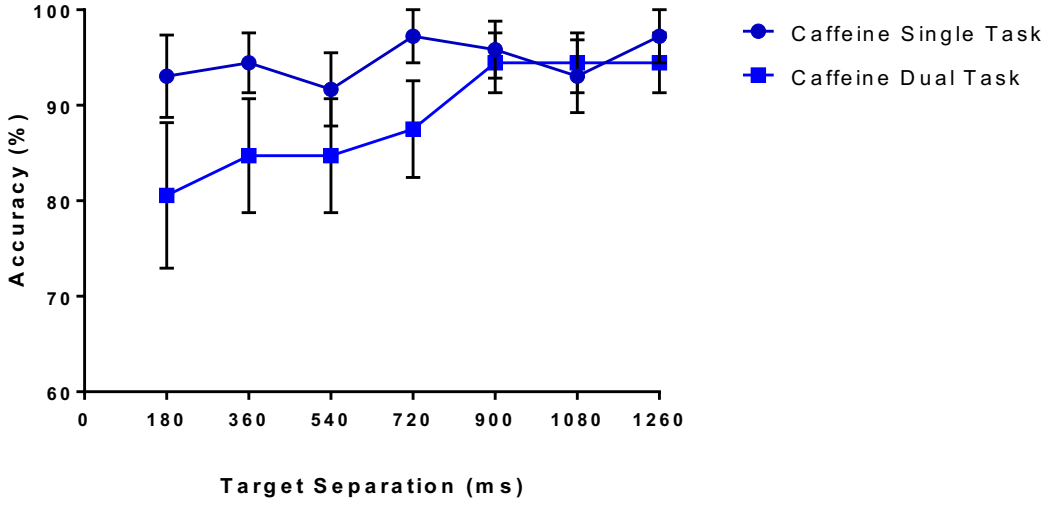

Figure 6.3a MS mean accuracy on single and dual tasks of the Rapid Serial Visual Presentation paradigm whilst on caffeine. Error bars represent standard error of the mean. The area of interest is the point of intersect between single and dual task result lines. This represents the "attentional blink", the time required to attend a primary target before disengaging and attending to a second target accurately. Under caffeine the attentional blink is 900 ms.

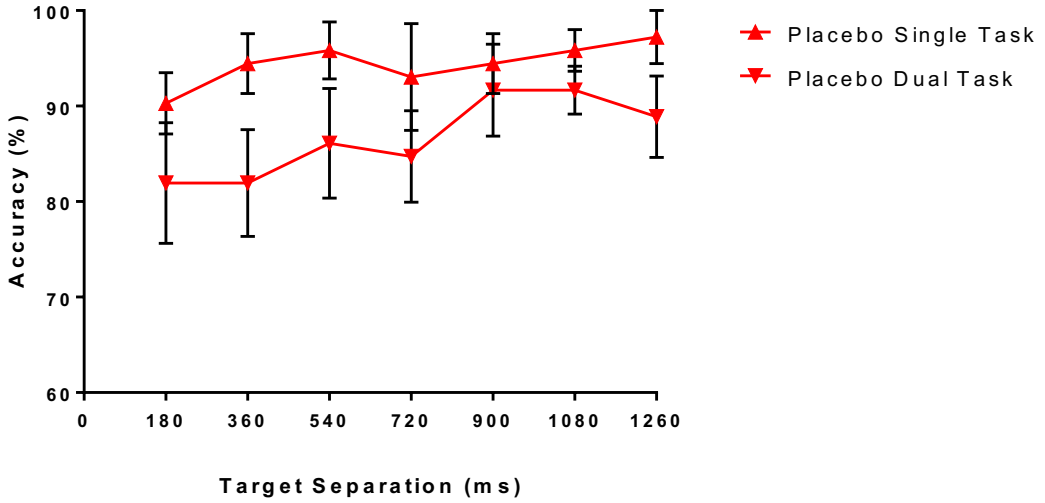

Figure 6.3b MS mean accuracy on single and dual tasks of the Rapid Serial Visual Presentation paradigm whilst on placebo. Error bars represent standard error of the mean. The attentional blink is 900 ms although it appears to worsen as target separation increases above this value.

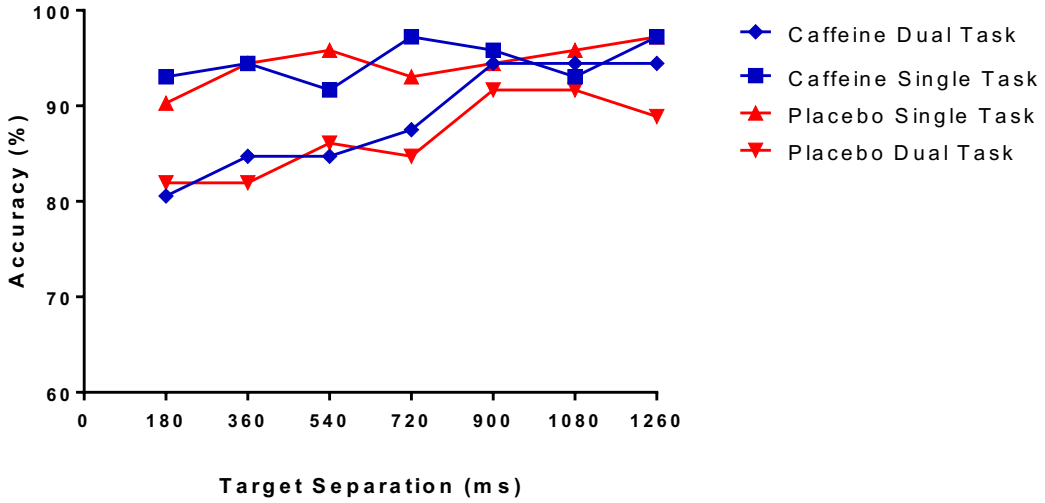

Figure 6.3c MS mean accuracy on single and dual tasks of the Rapid Serial Visual Presentation paradigm comparing caffeine with placebo. There is no statistical difference in the attention blink.

6.3.3 Executive attention

A paired-samples t-test was used to determine whether there was a statistically significant mean difference between caffeine versus placebo on ***Stroop reaction time*** scores. Data are mean ± standard deviation, unless otherwise stated.
The assumption of normality was not violated, as assessed by Shapiro-Wilk's test for neutral (p = 0.99), incongruent (p = 0.29) and total (p = 0.60) conditions.
There was no statistically significant change in reaction time during the neutral condition whilst on caffeine (874 ms ± 122) compared to placebo (869 ms ± 121), 5 ms, 95% CI [-45, 58], t(11) = 0.21, p = 0.84, d = 0.06.

There was no statistically significant change in reaction time during the incongruent condition whilst on caffeine (1006 ms ± 208) compared to placebo (1051 ms ± 272), -45 ms, 95% CI [-133, 42], t(11) = -1.14, p = 0.28, d = -0.33.

There was no statistically significant change in total Stroop reaction time whilst on caffeine (940 ms ± 162) compared to placebo (960 ms ± 192), -40 ms, 95% CI [-88, 48], t(11) = -0.652, p = 0.527, d = -0.03.

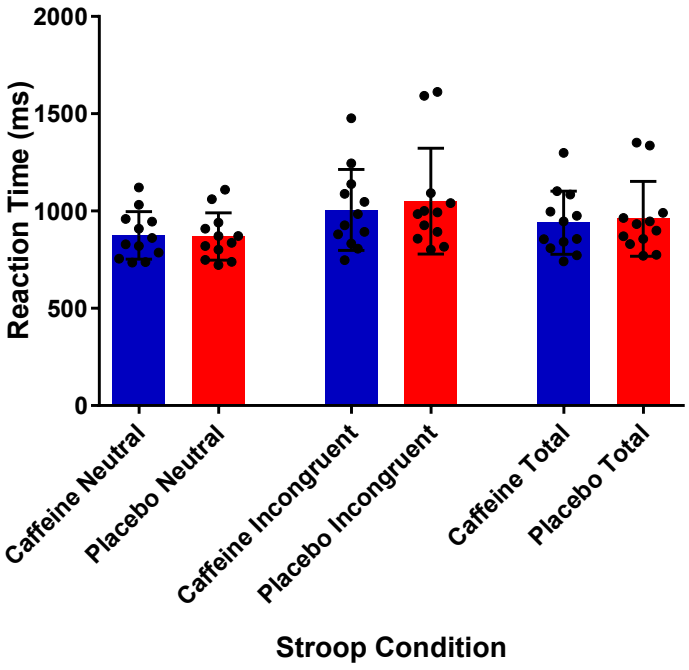

Figure 6.4 MS mean reaction time performance on the Stroop task. No significant difference was observed.

6.3.4 Walking while talking

A related samples Sign test was used to determine whether there was a statistically significant median difference between caffeine versus placebo on *walking while talking* times. Data are medians unless otherwise stated.

There was no statistically significant change in walking times on caffeine (23.0) compared to placebo (23.0) p = 0.77. There was no statistically significant change in walking while talking times on caffeine (37.7) compared to placebo (36.9) p = 1.00.

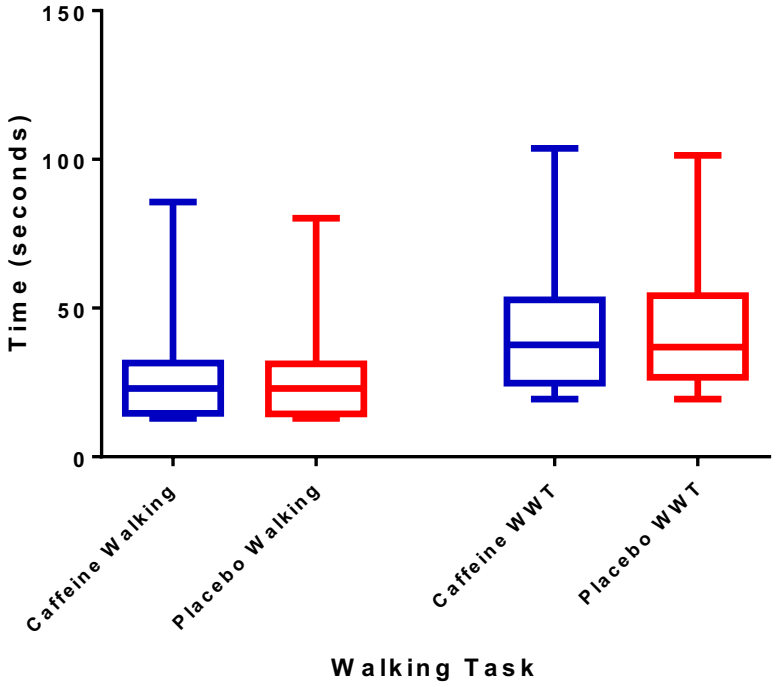

Figure 6.5 MS walking while talking task performance. Almost identical performance on caffeine and placebo.

6.3.5 Digit Span

A paired-samples t-test was used to determine whether there was a statistically significant mean difference between caffeine versus placebo on ***digit span score***s. Data are mean ± standard deviation, unless otherwise stated.

The assumption of normality was not violated, as assessed by Shapiro-Wilk's test for forward (p = 0.06), back (p = 0.39) and total (p = 0.64) conditions.

There was no statistically significant change in digit span during the forward condition whilst on caffeine (11.4 digits ± 2.4) compared to placebo (11.8 digits ± 2.4), -0.4 digits, 95% CI [-1.5, 0.7], t(11) = -0.81, p = 0.44, d = 0.23.

There was no statistically significant change in digit span during the backwards condition whilst on caffeine (8.1 digits ± 2.9) compared to placebo (8.8 digits ± 2.3), -0.7 digits, 95% CI [-1.9, 0.6], t(11) = -1.20, p = 0.26, d = 0.35.

There was no statistically significant change in total digit span whilst on caffeine (19.5 digits ± 4.8) compared to placebo (20.6 digits ± 4.3), -1.1 digits, 95% CI [-2.7, 0.5], t(11) = -1.52, p = 0.16, d = 0.44.

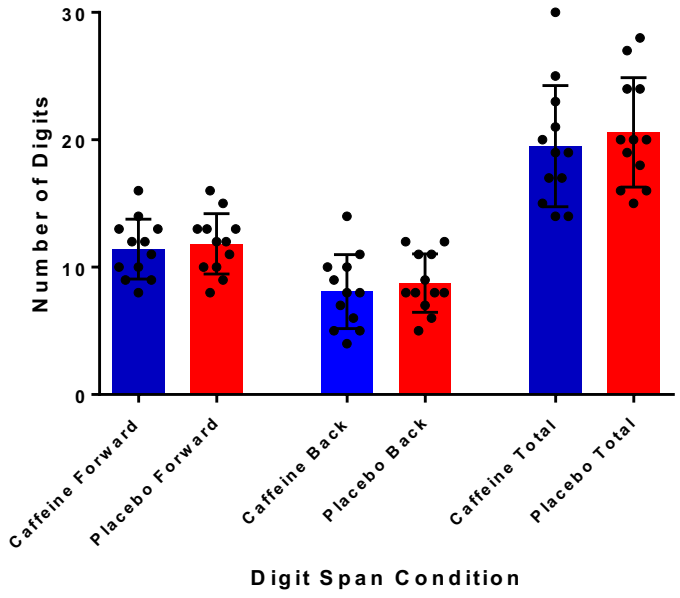

Figure 6.6 MS digit span performance. Error bars represent standard error of the mean. There was a non-significant worsening of digit span length by caffeine.

6.3.6 Correlations

A Pearson product-moment correlation coefficient was run to assess the relationship between choice reaction time and MoCA score. There was a moderate negative correlation between choice reaction time and MoCA score $r(11) = -0.64$, $p = 0.04$. As MoCA score increased, cognitive reactions times decreased i.e. were faster.

There was no other significant correlation between age, MoCA score, sex or habitual caffeine intake and any of the tests described above. There was no effect of intervention crossover order as a between subjects variable for any of the tests.

6.3.7 Sample size calculation

From the small sample size obtained in this pilot study, I have calculated the required sample size to power a definitive study. I conducted post hoc power analyses using G*Power (Faul et al., 2007) with power $(1 - \beta)$ set at 0.80 and $\alpha = 0.05$, two-tailed.

To test our hypotheses with statistical significance at the 0.05 level:

- alerting attention sample sizes would be N = 198, 20 and 28 for SRT, CRT and CogRT, respectively
- orienting attention sample sizes would be N = 89
- executive attention sample sizes would be N = 2182, 74 and 219 for congruent, incongruent and total Stroop, respectively
- digit span sample sizes would be N = 150, 66 and 43 for forward, backward and total digit span, respectively
- WWT sample sizes would be N = 214 and 1,962,509 for walking and WWT, respectively

6.4 Discussion

6.4.1 Promising results

This study investigated the effect of caffeine on each individual subtype of attention, in MS participants. As a pilot study the main aims were to assess suitability to progress to a larger study, including determining the required sample size to demonstrate adequate power and evaluating recruitment feasibility. The sample was underpowered for hypothesis testing and this limits the interpretation of inferential statistics. As expected, caffeine did not significantly improve performance on tasks testing any attentional network, real world task of attention or functional tasks of working memory, although trends were present. Below I discuss the feasibility of progressing to a definitive study and explore the trends in data analysis.

The low recruitment rate despite the high screening numbers, substantiate the difficulty in recruiting MS patients with cognitive problems into clinical trials. Similarly this was noted in Chapter 3 when trying to recruit dementia with Lewy body participants. Reassuringly only 1 participant was unable to tolerate caffeine withdrawal and was therefore withdrawn from the trial, prior to randomisation. This represents a fallout rate of 8% (1/13) which can be factored into future sample size calculations. This pilot study highlighted the principal impediment to recruitment as a high decline rate (44/64) for study involvement. Despite participants being recruited from a research database they were still reluctant to participate. Having discussed reasons for study participation decline with those who were screened, it is clear the most off putting features of the study relate to multiple attendances (4 in total) and difficulty with travel arrangements. The MS population is younger than other cohorts in this thesis and are often actively engaged in employment. Attendance at this study would therefore require taking 4 days annual leave or unpaid leave, which is clearly unattractive and much less than the £10 per day remuneration fee offered with the trial.

The alerting attention tests all show a non-statistically significant improvement with caffeine; the choice reaction time and cognitive reaction time demonstrate a moderate effect size according to Cohen (Cohen, 1988). As expected there is no suggestion of a difference in simple reaction time scores as this does not require significant cognitive processing and hence one would not expect caffeine to attenuate this response. The post hoc power analyses suggest the non-significance is due to the study being underpowered, as would be expected in a pilot study. If a future powered study demonstrated significance of intervention and the result effect size remained the same, this may yield a significant clinical benefit and improved quality of life, as it would offer a treatment for impaired attention for which no medications are currently licensed or recommended.

Executive attention, measured by the Stroop task, demonstrated a small effect on congruent condition but a much larger, moderate effect on incongruent condition. Once again the post hoc power analyses suggested inadequate power for significance due to low sample size. The differential between the effect sizes for congruent and incongruent conditions is suggestive of caffeine improving executive attention. In the Stroop task the congruent condition represents an automatic response and acts as an experiment control whilst the incongruent condition results represent the so called Stroop interference effect (Lovett, 2005) – a test of executive function. An attentional enhancer of the executive domain would therefore be expected to improve the incongruent condition in preference to the congruent condition, as is the case in this pilot study.

Amantadine, the only licenced treatment for MS fatigue is thought to mediate its effects as a dopamine agonist, it is unknown whether dopamine pathway function is sub-clinically low in MS patients. Caffeine has been proposed to mediate its alerting effects through dopamine up regulation (Brunye et al., 2010). Impaired executive attention will produce a significant burden of the life of MS suffers who are typically young as it can result in occupation loss from inability to complete complex but everyday tasks in a timely manner. A future study would be to solidify

the extent of dopaminergic activity in MS suffers and if this was established as low, future trials using dopaminergic medications would be warranted.

All tests were assessed for correlations to age, MoCA score, sex and habitual caffeine intake. The rationale being to tease out any relationships, which would benefit from a subgroup analysis or could be re-targeted in a future study. The only significant correlation was a slower Choice Reaction Time score with decreasing Montreal Cognitive Score. This is hardly surprising as a low MoCA score indicates cognitive decline, a significant component of this is known to be impaired processing speed and therefore one would expect this pattern not just for choice reaction time but in all the computerised attention tasks. The lack of other correlations is likely an indicator of inadequate power.

As expected there was no significant effect or trend towards caffeine having an effect on orienting attention. There was no effect or trend on walking while talking time, a real world task of attention. This is likely due to the task not being an adequate discriminator as a result of its relative simplicity. It is possible that a more taxing walking while talking paradigm, that stresses the attentional network, would be better suited to probe the effects of caffeine.

The digit span served as a test of working memory, whilst again not significant, did demonstrate a trend towards caffeine having a negative effect, reducing forward, backward and reliable digit span. This is surprising and counter intuitive to the notion of caffeine improving attention, which ought to improve the functional capacity of working memory. The reason for this is not clear and at odds with other published studies examining a similar effect in healthy participants (Borota et al., 2014, Nehlig, 2010), although these studies did not fully withdraw participants prior to intervention. A possible explanation for my results is the association of anxiety and restlessness induced by adrenergic stimulation by caffeine ingestion in naïve or fully withdrawn individuals. Caffeine is known to induce cerebral vasoconstriction (Field et al., 2003) and it may divert blood flow

away from salient cerebral areas involved in working memory, thus reducing its function. It is therefore advantageous to include it in a larger trial to assess whether attention is enhanced at the expense of another cognitive domain.

6.4.2 Limitations

An obvious limitation for inferential statistics described here is the small sample size of this pilot study. The poor ratio of potential participant screened to trial participation will inevitably result in participant bias. Participants may have been more inclined to participate if they already felt they received a cognitive benefit from caffeine and likewise screened participants who declined might have been non or low caffeine consumers who derived little benefit from this drug.

It would have been useful to quantify fatigue in participants to allow assessment of whether subjective scores of fatigue correlated with impaired attention and if response to caffeine could be stratified by fatigue. Obtaining baseline and post treatment fatigue scores is a feature, which can be built into to future studies.

The walking while talking task appears to be a poor discriminator as a real world task of attention. Given the trend of improvement following caffeine in alerting and orienting attention, one would expect a similar trend on the walking while talking task. The lack of improvement could be due to a genuine lack of effect or more likely, the parameters of the task did not allow an effect to be uncovered. The walking while talking task was too easy and did not sufficiently stress attentional capacity adequately, therefore participants could perform the task optimally rendering attentional enhancers redundant.

6.4.3 Conclusion

This study has demonstrated a trend toward caffeine having a beneficial effect on alerting and orienting attention as expected and unexpectedly worsening

working memory. The pilot study provides justification for a larger adequately powered trial.

I would propose a trial of 80 participants to achieve adequate power to test the hypothesis: acute caffeine ingestion will improve attention in the alerting and executive domain in people with MS. This recruitment target takes into account an expected 8% drop out.

Chapter 7

General Discussion

The experiments described in this thesis were designed to explore the influence of caffeine on attention, especially with respect to selective attentional network enhancement. Over the years several attentional models have been proposed with the most contemporary being Posner's trinity of attentional networks, *alerting*, *orienting* and *executive*, integrating both neuroanatomical pathways and psychological function (Petersen and Posner, 2012). The therapeutic effects of caffeine were modelled on healthy elderly participants and cohorts with impaired attention comprising dementia with Lewy bodies (DLB), Parkinson's disease (PD) and multiple sclerosis (MS). The remainder of this chapter will précis key concepts identified within this thesis, describe limitations, suggest future avenues of scientific endeavour and draw conclusions.

7.1 Critical flaws in the existing body of research

There is over a hundred years of research investigating the effect of caffeine on attention, with consensus of its beneficial psychostimulant properties purported by recent reviews (Einother and Giesbrecht, 2013, Nehlig, 2016). This is countered by a minority of sceptics (James, 2014, Rogers et al., 2013) who support the *caffeine withdrawal reverse hypothesis*. This asserts caffeine consumed prior to full withdrawal, simply acts to ameliorate the fatiguing effects of withdrawal itself rather than produce an overall, net improvement in cognitive function.

Following cessation, caffeine withdrawal peaks after 1 to 2 days and can last up to 9 days. There are a dearth of studies, which use either caffeine naïve or fully withdrawn participants and over 95% of all studies use a withdrawal period of 48 hours or less, leaving their validity in doubt. Of the few placebo controlled trials

assessing the effect of caffeine on attention in participants who had been withdrawn at least 96 hours, there is no clear improvement in attention except in those who were sleep deprived (Kamimori et al., 2015). Studies with an adequate withdrawal period were performed exclusively in healthy, young populations and did not systematically examine if attention was enhanced by caffeine i.e. of the trinity of attentional networks, experimental tasks only assessed one or two of the three attentional domains.

A key variable when performing caffeine research is determining the dose of the intervention. Caffeine doses as low as 20mg and as high as 800mg/day have been trialled but few studies have performed head to head comparisons of the effect of different doses on the same cohort using the same attention tests (Lieberman et al., 1987, Kamimori et al., 2015). It is perhaps because of the assumption that the greater the caffeine dose, the greater the attentional enhancement. This is in juxtaposition to the Yerkes-Dodson law, which proposes maximal stimulation is not the same as optimal stimulation (Yerkes and Dodson, 1908). Instead attention performance conforms to an inverted U shape where increases in arousal are associated with an increase in performance up to an optimum point, following which higher levels of arousal only impair performance. It is therefore conceivable a low or moderate dose may be more effective than high dose caffeine.

The experiments contained within this thesis are novel in the field of caffeine research as they combined (i) a battery of tests systematically developed to align with each of the three attentional network domains; (ii) participants whom were fully caffeine withdrawn prior to testing; (iii) novel subject groups including healthy elderly and those with medically acquired impaired attention. The combination of the characteristics described above ensured robust data was obtained and gave validity to interpretation of findings.

7.2 Caffeine does not enhance attention in healthy elderly participants

Healthy elderly participants were tested on tasks assessing attention at both habitual caffeine intake levels of 63mg (i.e. a normal coffee) and a moderate dose of 100mg (Chapters 4,5 and 6). In both cases caffeine failed to enhance any domain of attention when compared to placebo. This is in stark contrast to the majority of published caffeine trials but is in keeping with the few trials that employed an adequate caffeine withdrawal period prior to testing.

There are two deductions possible from this finding. Healthy elderly people by definition have normal attention, for their age. Therefore according to the Yerkes-Dawson law, their attention is not amenable to therapeutic enhancement by caffeine or indeed any other psychoactive prescription as it is already performing optimally. Another possibility is that the withdrawal reversal hypothesis is true. This study adds to the small body of research, which demonstrates no effect of caffeine on attention if a long enough withdrawal period is utilised prior to testing.

The only way to delineate between the two deductions was to test the effect of caffeine in cohorts of participants with attention deficits. The experiments were therefore repeated in participants with neurological conditions, which consequentially impaired their attention.

7.3 Caffeine may exert its main effect through dopaminergic pathway enhancement

PD participants were tested using the same experiment design as for healthy elderly participants, with a caffeine dose of 100mg (Chapter 5). Whilst PD is classically considered in terms of its motor features, cognitive impairment affecting attention, memory and executive function are increasingly recognised, (Adler and Thorpy, 2005) in association with excessive daytime somnolence. The pathophysiological hallmark of PD is the loss of dopaminergic neurons (Lee and

Trojanowski, 2006). Caffeine is proposed to elicit an effect through dopaminergic pathway enhancement via inhibition of the descending GABAergic system or through enhancement of the ascending brainstem aminergic system. Adenosine receptors are co-localised with dopaminergic D2 receptors and antagonise their function (Ferre et al., 2008). Caffeine antagonises adenosine receptors and will consequentially inhibit the somnolent effect of adenosine receptors on dopamine D2 receptor pathways, making it in theory an ideal wakefulness promoting medication.

There was no improvement in any attentional domain in PD participants following caffeine ingestion. A possible physiological explanation for the negative study is the loss of dopamine production in PD, which results in dopamine receptors loss rendering the co-localised adenosine receptors redundant and the effect of caffeine impotent.

MS participants were also tested on attention tasks following 100mg caffeine administration as part of a pilot study (Chapter 6). Fatigue is a common and debilitating symptom of MS, affecting between 65 and 92% (Branas et al., 2000). The pathophysiology of mental fatigue in MS has not been elucidated but it is increasingly postulated that disruption of the normal hypothalamic-pituitary axis and neurotransmitter pathways are the underlying basis of symptoms (Bol et al., 2010, Lucchinetti et al., 2011, Filippi et al., 2002).

Dopaminergic networks have been shown to be involved in fatigue, which has been proposed to occur due to failure of the non-motor function of the basal ganglia, termed the dopamine imbalance theory (Dobryakova et al., 2015). Disruption and/or dysfunction of the thalamo-striato-cortical network is proposed as the anatomical pathway responsible for fatigue resulting in neurotransmitter disruption between the striatum and prefrontal cortex (Alexander and Crutcher, 1990). Dysfunction of the thalamo-striato-cortical pathways correlates with impairment in the alerting and executive attentional networks. Fatigue is poorly defined but is in part a subjective manifestation of decreased attention and was therefore considered amenable to attentional enhancement.

As a pilot study, the sample size was underpowered and consequentially did not produce any significant results, however, analysis did reveal trends within the data. Alerting attention as assessed by the cognitive reaction time and executive attention assessed by the Stroop task, both demonstrated non-significant improvement following caffeine ingestion, with moderate effect size according to Cohen (Cohen, 1992). Whilst this data should be interpreted with caution, it points towards the possibility of caffeine enhancing attention in cohorts with intrinsically impaired attention provided they have intact dopaminergic pathways. Of course a higher powered study would be required to confirm this hypothesis.

7.4 Selective attentional impairments in PD participants but not DLB participants when compared to healthy aged matched controls

Until now a singular study has not systematically assessed each domain of attention (or equivalent if not using Posner's model) in PD or DLB participants with aged matched healthy controls for comparison (Chapters 3 and 5). As expected DLB participants performed poorly across the board compared to controls. By contrast PD participants, who had cognitive impairment as part of their recruitment criteria, demonstrated selective deficits in executive and to a lesser degree orienting attention.

Interestingly performance of PD participants in a real world task of attention, the walking while talking task, demonstrated no difference in walking time but a significant difference in walking while talking time, a surrogate marker of falls (LaPointe et al., 2010). The disparity between the 2 test indices suggest motor speed is not a factor of the significant result, instead it is attributable, at least in part to impaired attention in PD. Having systematically assessed each attentional domain, it can be deduced impaired walking while talking time corresponds best with impaired executive attention. Phase 2 clinical trials have demonstrated promise for Rivastigmine as a remedy for gait instability. It acts as a cholinesterase inhibitor, increasing cholinergic transmission which enhances orienting attention (Henderson

et al., 2016). My data confers cholinesterase inhibitors may be of some benefit at improving gait stability but optimising dopaminergic pathways will be of the greatest benefit, however, this may not be feasible in this population.

7.5 Limitations

The optimal caffeine dose to enhance attention is not known. I opted for a moderate dose of 100mg caffeine as the intervention, which is greater than found in foodstuffs but smaller than any other trial, which fully withdrew participants prior to testing (Smith et al., 2013, Rogers et al., 2005, Kamimori et al., 2015, Judelson et al., 2005). Whilst these other studies exclusively tested healthy participants, my subject groups included the elderly and patient groups. I therefore adopted a more cautious approach due to the increased risk of side effects. It could be argued the caffeine dose used was simply too low, however, some studies have shown a beneficial effect from caffeine with doses as low as 20mg, although they did not fully withdraw their participants (Lieberman et al., 1987). Inter-individual variability in response to caffeine is recognised but difficult quantify. ADORA2A genetic polymorphism of the adenosine receptor is recognised as significant factor in caffeine sensitivity (Cornelis et al., 2007). In this negative trial a subgroup analysis of those with and without this mutation may have yielded interesting results.

A recurring criticism of clinical research is the publication of underpowered data, which is liable to type I error (Carlisle et al., 2015). Where appropriate I have included a post hoc sample size calculation. Statistically, it would be most appropriate to present the study power result but comparing the required sample size for a powered study, to the actual sample size is an easier indicator to gauge. Clearly a greater sample size would have produced better study power, more reliable data and given stronger validity to deductions. The reality of clinical research means patient research databases are smaller and much harder to recruit

from than healthy participant databases, as evidenced by the recruitment flow charts in each data chapter.

The use of research registers to identify potential participants and the selective nature of volunteering to participate led to inherent selection bias. As this was a caffeine study, it is likely participants who did not feel any beneficial effect from caffeine, avoided participation and this is reflected to a degree by the lack of naïve caffeine consumer participation.

Attention assessment was subdivided according to Posner-Petersen model and an established and validated neuropsychological test was chosen to test each of alerting, orienting and executive domains. There are dozens of potential tests that could be used to assess each attentional domain with my final choice based on ease of use and availability as a customisable computerised test. Posner's research group have developed the attentional network task (Fan et al., 2002) which is a singular computerised test designed to assess all attentional domains. I did not use this as the parameters and instructions were not customisable and I was concerned participants with dementia or other forms of cognitive impairment would find it difficult to understand and execute. The advantage of Posner's task is the large frequency of trials, which can be performed in a short space of time. Testing fatigue is always a concern when designing an experiment and collecting as much data as possible only serves to improve analysis reliability.

It is important to recognise the limitations of the Posner-Petersen model and consider the subsequent effect on trial design and result interpretation. The neuropsychological tests represent assessment of more than a singular, unitary anatomical pathway as alluded to by Posner. Had I chosen a different attention model by Yu and Dayan or Corbetta for example, the neuropsychology test battery would have been very different and potentially this would have yielded different results with a different conclusion.

A protocol amendment occurred between the first data chapter 3 and subsequent data chapters 4, 5 and 6. The main change was an intervention change

from coffee containing 63mg of caffeine to decaffeinated coffee with the addition of 100mg crushed caffeine tablets. With the change in intervention came a change in blinding procedure, moving from double blind to single blind. This reduces the validity of the study as the investigator running the experiment now knows which intervention the participant has received which may have subconsciously affected the interaction with them. The cost for a pharmacy to make placebo and caffeine tablets in the same manner was not possible with the study budget. I have now discovered empty cellulose capsules are available for purchase from an online retailer; these cost very little and can be easily made into batches of caffeine and placebo, and subsequently blinded to both participant and researcher.

During the caffeine withdrawal period prior to intervention, I monitored caffeine abstinence by a daily caffeine consumption survey. At least some participants completed this honestly as it resulted in them being withdrawn from the trial. The ideal monitoring of withdrawal compliance would be through measurement of caffeine salivary levels (Smith et al., 2013), I was limited from using this due to financial constraints. A compromise would be to take caffeine saliva samples but not process them. This might prompt participants to ensure they completed caffeine monitoring honestly and has been adopted as a strategy by other researchers (personal correspondence with Gary Christopher).

This was a cross over trial, which fully withdrew participants from caffeine at the start before randomly allocating the participant to caffeine or placebo followed by the alternate intervention the *following* day. Whilst there was no effect of intervention crossover order as a between subjects variable for any of the tests, it is still possible participants who received caffeine first (on day 8) followed by placebo (on day 9) may have been in a state of withdrawal during the second day of testing. The only way to negate this was to space testing between caffeine and placebo by one week, extending the participant caffeine abstinence period to 15 days. This was thought to be too onerous for participants and had the potential for computerised test practice effects to be lost on latter testing sessions, causing further confounds in the data.

A remit of this research was to objectively assess attention and the effect of caffeine on it and I therefore used quantitative neuropsychological tests and purposefully avoided subjective measures of mood or attention. Given I have negative findings it would have been interesting to assess for a disparity between subjective feelings of improved attention with objective measures of attention. A sleep assessment in the health elderly trial would allow a subgroup analysis to assess if sleep duration/deprivation correlated with response to caffeine. Likewise in the MS cohort it would be interesting to correlated fatigue scores with attention and assess if they are differentially enhanced by caffeine.

Furthermore I have proposed caffeine elicits an effect through dopaminergic pathway enhancement via inhibition of the descending GABAergic system or through enhancement of the ascending brainstem aminergic system. Dopamine is intimately linked to reward as so pre and post caffeine ingestion mood assessments would potentially add weight to this assertion. These assessments are brief and easy to administer in any future studies. It would have been useful to assess for mesolimbic symptoms such as depression or anxiety via questionnaire and assess whether this correlated to caffeine consumption. A negative correlation would support the hypothesis of caffeine consumption being related to reward mechanisms. Another marker of dopamine system functioning is spontaneous eye blink rate. This has been shown to correlate to striatal dopamine levels (Dreisbach et al., 2005) and is clinically observed as high blink rates in schizophrenics and low blink rates in Parkinson's disease (Swerdlow et al., 2003). I could have taken pre and post measures of eye blink rate to assess if dopaminergic stimulation was a prominent factor in performance.

7.6 Future directions

There may be a role for caffeine to enhance attention in health elderly participants when they are sleep deprived. As discussed above when attention is

optimal as is typically the case in healthy participants, enhancement is likely to be futile. However, under certain environmental conditions that stress the attentional system of healthy people, such as sleep deprivation, their usually optimal attention will become sub-optimal and therefore amenable to attentional enhancers.

The pilot study involving MS participants showed great promise and it would be interesting to repeat this experiment as a powered study, although recruitment in this population is difficult and may require a multicentre approach. It would also be worthwhile investigating the effect of caffeine in patient populations with intact dopaminergic pathways but pathology leading to impaired attention such as Alzheimer's disease and certain forms of acquired brain injury for instance encephalitis and traumatic brain injury. These conditions lack symptomatic treatments and setting up a caffeine study would be safe, supported by patient groups and financially feasible.

I hypothesize caffeine elicits its main effect through dopaminergic stimulation and this may relate not just to attention enhancement but also mood effects, which facilitate tolerance and dependence. Combining neuropsychometry tests of attention with functional MRI imaging would allow better characterisation of the brain areas and associated neurotransmitter networks involved.

7.7 Conclusions

Within this thesis I have judiciously analysed the body of research regarding the effects of caffeine on attention. Despite a critical mass of papers supporting the beneficial enhancing effects of caffeine, a minority of authors supported the caffeine withdrawal reversal hypothesis. This proposes caffeine's attention enhancing effects are the result of reversing caffeine withdrawal, caused by a flawed study design with an inadequate withdrawal period prior to testing. The few caffeine studies with

an adequate withdrawal period have exclusively tested young healthy participants and have not demonstrated a clear beneficial effect.

I designed an experiment to test whether caffeine improved attention in healthy elderly, DLB, PD and MS participants following a full caffeine withdrawal period prior to testing. Attention tests were matched according to each of Posner's trinity of attentional domains, alerting, orienting and executive attention. In addition I included the walking while talking task, a real world test of attention.

Healthy elderly participants did not have their attention enhanced by caffeine, most likely because their attention was already functioning at an optimal level.

Compared to age matched controls DLB participants displayed widespread deficits in attention whilst PD participants were mainly impaired in executive attention, which is dependent on dopaminergic network function. Neither of these participant groups had their attention enhanced by caffeine and I propose this is due to caffeine eliciting its main effect through dopamine up regulation. In both these conditions there is loss of dopaminergic neurons, dopamine receptors and co-localised adenosine receptors and therefore endogenous dopamine enhancement is not possible, preventing caffeine from generating an attention enhancing effect.

MS participants were tested as part of a pilot study and demonstrated exciting non-significant data trends. There was a moderate effect size towards an improvement in alerting and executive attention. These non-significant results should be viewed with caution but do warrant further investigation with a powered study.

This thesis has highlighted the misconception of caffeine as an attention enhancer for healthy individuals and given weight to the caffeine withdrawal reversal hypothesis. Until now caffeine has not been trialled as an acute attention enhancer in populations with neurological disease. Whilst the results are negative in conditions with dopaminergic neuron loss, caffeine shows promise as an enhancer in conditions with impaired attention but intact dopamine networks, an exciting avenue for future research.

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